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COMPOSITIONS AND METHODS FOR USE IN RECOMBINATIONAL  
CLONING OF NUCLEIC ACIDS

Abstract:

The present invention relates generally to compositions and methods for use in recombinational cloning of nucleic acid molecules. In particular, the invention relates to nucleic acid molecules encoding one or more recombination sites or portions thereof, to nucleic acid molecules comprising one or more of these recombination site nucleotide sequences and optionally comprising one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides using the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof. The invention also relates to the use of these compositions in methods for recombinational cloning of nucleic acids, in vitro and in vivo, to provide chimeric DNA molecules tha  
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t have particular characteristics and/or DNA segments.

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<b>(54) Title:</b> COMPOSITIONS AND METHODS FOR USE IN RECOMBINATIONAL CLONING OF NUCLEIC ACIDS  <b>(57) Abstract</b>  The present invention relates generally to compositions and methods for use in recombinational cloning of nucleic acid molecules. In particular, the invention relates to nucleic acid molecules encoding one or more recombination sites or portions thereof, to nucleic acid molecules comprising one or more of these recombination site nucleotide sequences and optionally comprising one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides using the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof. The invention also relates to the use of these compositions in methods for recombinational cloning of nucleic acids, <i>in vitro</i> and <i>in vivo</i> , to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.		



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## Compositions and Methods for Use in Recombinational Cloning of Nucleic Acids

### BACKGROUND OF THE INVENTION

#### *Field of the Invention*

The present invention relates generally to recombinant DNA technology. More particularly, the present invention relates to compositions and methods for use in recombinational cloning of nucleic acid molecules. The invention relates specifically to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences. The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides and RNAs encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments. More particularly, the antibodies of the invention may be used to identify and/or purify proteins or fusion proteins encoded by the nucleic acid molecules or vectors of the invention, or to identify and/or purify the nucleic acid molecules of the invention.

*Related Art*

*Site-specific recombinases.* Site-specific recombinases are proteins that are present in many organisms (e.g. viruses and bacteria) and have been characterized to have both endonuclease and ligase properties. These recombinases (along with associated proteins in some cases) recognize specific sequences of bases in DNA and exchange the DNA segments flanking those segments. The recombinases and associated proteins are collectively referred to as "recombination proteins" (see, e.g., Landy, A., *Current Opinion in Biotechnology* 3:699-707 (1993)).

Numerous recombination systems from various organisms have been described. See, e.g., Hoess *et al.*, *Nucleic Acids Research* 14(6):2287 (1986); Abremski *et al.*, *J. Biol. Chem.* 261(1):391 (1986); Campbell, *J. Bacteriol.* 174(23):7495 (1992); Qian *et al.*, *J. Biol. Chem.* 267(11):7794 (1992); Araki *et al.*, *J. Mol. Biol.* 225(1):25 (1992); Maeser and Kahnmann *Mol. Gen. Genet.* 230:170-176 (1991); Esposito *et al.*, *Nucl. Acids Res.* 25(18):3605 (1997).

Many of these belong to the integrase family of recombinases (Argos *et al.* *EMBO J.* 5:433-440 (1986); Voziyanov *et al.*, *Nucl. Acids Res.* 27:930 (1999)). Perhaps the best studied of these are the Integrase/*att* system from bacteriophage  $\lambda$  (Landy, A. *Current Opinions in Genetics and Devel.* 3:699-707 (1993)), the Cre/*loxP* system from bacteriophage P1 (Hoess and Abremski (1990) In *Nucleic Acids and Molecular Biology*, vol. 4. Eds.: Eckstein and Lilley, Berlin-Heidelberg: Springer-Verlag; pp. 90-109), and the FLP/FRT system from the *Saccharomyces cerevisiae* 2  $\mu$  circle plasmid (Broach *et al.* *Cell* 29:227-234 (1982)).

Backman (U.S. Patent No. 4,673,640) discloses the *in vivo* use of  $\lambda$  recombinase to recombine a protein producing DNA segment by enzymatic site-specific recombination using wild-type recombination sites *attB* and *attP*.

Hasan and Szybalski (*Gene* 56:145-151 (1987)) discloses the use of  $\lambda$  Int recombinase *in vivo* for intramolecular recombination between wild type *attP* and *attB* sites which flank a promoter. Because the orientations of these sites are

inverted relative to each other, this causes an irreversible flipping of the promoter region relative to the gene of interest.

Palazzolo *et al.* *Gene* 88:25-36 (1990), discloses phage lambda vectors having bacteriophage  $\lambda$  arms that contain restriction sites positioned outside a cloned DNA sequence and between wild-type *loxP* sites. Infection of *E. coli* cells that express the Cre recombinase with these phage vectors results in recombination between the *loxP* sites and the *in vivo* excision of the plasmid replicon, including the cloned cDNA.

Pósfai *et al.* (*Nucl. Acids Res.* 22:2392-2398 (1994)) discloses a method for inserting into genomic DNA partial expression vectors having a selectable marker, flanked by two wild-type FRT recognition sequences. FLP site-specific recombinase as present in the cells is used to integrate the vectors into the genome at predetermined sites. Under conditions where the replicon is functional, this cloned genomic DNA can be amplified.

Bebee *et al.* (U.S. Patent No. 5,434,066) discloses the use of site-specific recombinases such as Cre for DNA containing two *loxP* sites for *in vivo* recombination between the sites.

Boyd (*Nucl. Acids Res.* 21:817-821 (1993)) discloses a method to facilitate the cloning of blunt-ended DNA using conditions that encourage intermolecular ligation to a dephosphorylated vector that contains a wild-type *loxP* site acted upon by a Cre site-specific recombinase present in *E. coli* host cells.

Waterhouse *et al.* (WO 93/19172 and *Nucleic Acids Res.* 21 (9):2265 (1993)) disclose an *in vivo* method where light and heavy chains of a particular antibody were cloned in different phage vectors between *loxP* and *loxP 511* sites and used to transfect new *E. coli* cells. Cre, acting in the host cells on the two parental molecules (one plasmid, one phage), produced four products in equilibrium: two different cointegrates (produced by recombination at either *loxP* or *loxP 511* sites), and two daughter molecules, one of which was the desired product.

Schlake & Bode (*Biochemistry* 33:12746-12751 (1994)) discloses an *in vivo* method to exchange expression cassettes at defined chromosomal locations, each flanked by a wild type and a spacer-mutated FRT recombination site. A

double-reciprocal crossover was mediated in cultured mammalian cells by using this FLP/FRT system for site-specific recombination.

Hartley *et al.* (U.S. Patent No. 5,888,732) disclose compositions and methods for recombinational exchange of nucleic acid segments and molecules, including for use in recombinational cloning of a variety of nucleic acid molecules *in vitro* and *in vivo*, using a combination of wildtype and mutated recombination sites and recombination proteins.

**Transposases.** The family of enzymes, the transposases, has also been used to transfer genetic information between replicons. Transposons are structurally variable, being described as simple or compound, but typically encode the recombinase gene flanked by DNA sequences organized in inverted orientations. Integration of transposons can be random or highly specific. Representatives such as Tn7, which are highly site-specific, have been applied to the *in vivo* movement of DNA segments between replicons (Lucklow *et al.*, *J. Virol.* 67:4566-4579 (1993)).

Devine and Boeke *Nucl. Acids Res.* 22:3765-3772 (1994), discloses the construction of artificial transposons for the insertion of DNA segments, *in vitro*, into recipient DNA molecules. The system makes use of the integrase of yeast TY1 virus-like particles. The DNA segment of interest is cloned, using standard methods, between the ends of the transposon-like element TY1. In the presence of the TY1 integrase, the resulting element integrates randomly into a second target DNA molecule.

**Recombination Sites.** Also key to the integration/recombination reactions mediated by the above-noted recombination proteins and/or transposases are recognition sequences, often termed "recombination sites," on the DNA molecules participating in the integration/recombination reactions. These recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by the recombination proteins during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.*

5:521-527 (1994). Other examples of recognition sequences include the *attB*, *attP*, *attL*, and *attR* sequences which are recognized by the recombination protein  $\lambda$  Int. *attB* is an approximately 25 base pair sequence containing two 9 base pair core-type Int binding sites and a 7 base pair overlap region, while *attP* is an approximately 240 base pair sequence containing core-type Int binding sites and arm-type Int binding sites as well as sites for auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.* 3:699-707 (1993); see also U.S. Patent No. 5,888,732, which is incorporated by reference herein.

10        **DNA cloning.** The cloning of DNA segments currently occurs as a daily routine in many research labs and as a prerequisite step in many genetic analyses. The purpose of these clonings is various, however, two general purposes can be considered: (1) the initial cloning of DNA from large DNA or RNA segments (chromosomes, YACs, PCR fragments, mRNA, etc.), done in a relative handful  
15 of known vectors such as pUC, pGem, pBlueScript, and (2) the subcloning of these DNA segments into specialized vectors for functional analysis. A great deal of time and effort is expended both in the transfer of DNA segments from the initial cloning vectors to the more specialized vectors. This transfer is called subcloning.

20        The basic methods for cloning have been known for many years and have changed little during that time. A typical cloning protocol is as follows:

- (1) digest the DNA of interest with one or two restriction enzymes;
  - (2) gel purify the DNA segment of interest when known;
  - (3) prepare the vector by cutting with appropriate restriction  
25 enzymes, treating with alkaline phosphatase, gel purify etc., as appropriate;
  - (4) ligate the DNA segment to the vector, with appropriate controls to eliminate background of uncut and self-ligated vector;
  - (5) introduce the resulting vector into an *E. coli* host cell;
  - (6) pick selected colonies and grow small cultures overnight;
  - (7) make DNA minipreps; and
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(8) analyze the isolated plasmid on agarose gels (often after diagnostic restriction enzyme digestions) or by PCR.

The specialized vectors used for subcloning DNA segments are functionally diverse. These include but are not limited to: vectors for expressing nucleic acid molecules in various organisms; for regulating nucleic acid molecule expression; for providing tags to aid in protein purification or to allow tracking of proteins in cells; for modifying the cloned DNA segment (*e.g.*, generating deletions); for the synthesis of probes (*e.g.*, riboprobes); for the preparation of templates for DNA sequencing; for the identification of protein coding regions; for the fusion of various protein-coding regions; to provide large amounts of the DNA of interest, *etc.* It is common that a particular investigation will involve subcloning the DNA segment of interest into several different specialized vectors.

As known in the art, simple subclonings can be done in one day (*e.g.*, the DNA segment is not large and the restriction sites are compatible with those of the subcloning vector). However, many other subclonings can take several weeks, especially those involving unknown sequences, long fragments, toxic genes, unsuitable placement of restriction sites, high backgrounds, impure enzymes, *etc.* Subcloning DNA fragments is thus often viewed as a chore to be done as few times as possible.

Several methods for facilitating the cloning of DNA segments have been described, *e.g.*, as in the following references.

Ferguson, J., *et al. Gene* 16:191 (1981), discloses a family of vectors for subcloning fragments of yeast DNA. The vectors encode kanamycin resistance. Clones of longer yeast DNA segments can be partially digested and ligated into the subcloning vectors. If the original cloning vector conveys resistance to ampicillin, no purification is necessary prior to transformation, since the selection will be for kanamycin.

Hashimoto-Gotoh, T., *et al. Gene* 41:125 (1986), discloses a subcloning vector with unique cloning sites within a streptomycin sensitivity gene; in a streptomycin-resistant host, only plasmids with inserts or deletions in the dominant sensitivity gene will survive streptomycin selection.

Accordingly, traditional subcloning methods, using restriction enzymes and ligase, are time consuming and relatively unreliable. Considerable labor is expended, and if two or more days later the desired subclone can not be found among the candidate plasmids, the entire process must then be repeated with alternative conditions attempted. Although site specific recombinases have been used to recombine DNA *in vivo*, the successful use of such enzymes *in vitro* was expected to suffer from several problems. For example, the site specificities and efficiencies were expected to differ *in vitro*; topologically linked products were expected; and the topology of the DNA substrates and recombination proteins was expected to differ significantly *in vitro* (see, e.g., Adams *et al*, *J. Mol. Biol.* 226:661-73 (1992)). Reactions that could go on for many hours *in vivo* were expected to occur in significantly less time *in vitro* before the enzymes became inactive. In addition, the stabilities of the recombination enzymes after incubation for extended periods of time in *in vitro* reactions was unknown, as were the effects of the topologies (*i.e.*, linear, coiled, supercoiled, etc.) of the nucleic acid molecules involved in the reaction. Multiple DNA recombination products were expected in the biological host used, resulting in unsatisfactory reliability, specificity or efficiency of subcloning. Thus, *in vitro* recombination reactions were not expected to be sufficiently efficient to yield the desired levels of product.

Accordingly, there is a long felt need to provide an alternative subcloning system that provides advantages over the known use of restriction enzymes and ligases.

## SUMMARY OF THE INVENTION

The present invention relates to nucleic acid molecules encoding one or more recombination sites or one or more partial recombination sites, particularly *attB*, *attP*, *attL*, and *attR*, and fragments, mutants, variants and derivatives thereof. The invention also relates to such nucleic acid molecules comprising one or more of the recombination site nucleotide sequences or portions thereof and one or more additional physical or functional nucleotide sequences, such as those

encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (*e.g.*, one or more promoters, enhancers, or repressors), one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide (*e.g.*, GST, His<sub>6</sub> or thioredoxin), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more desired proteins or peptides encoded by a gene or a portion of a gene, and one or more 5' or 3' polynucleotide tails (particularly a poly-G tail). The invention also relates to such nucleic acid molecules wherein the one or more recombination site nucleotide sequences is operably linked to the one or more additional physical or functional nucleotide sequences.

The invention also relates to primer nucleic acid molecules comprising the recombination site nucleotide sequences of the invention (or portions thereof), and to such primer nucleic acid molecules linked to one or more target-specific (*e.g.*, one or more gene-specific) primer nucleic acid sequences. Such primers may also comprise sequences complementary or homologous to DNA or RNA sequences to be amplified, *e.g.*, by PCR, RT-PCR, etc. Such primers may also comprise sequences or portions of sequences useful in the expression of protein genes (ribosome binding sites, localization signals, protease cleavage sites, repressor binding sites, promoters, transcription stops, stop codons, etc.). Said primers may also comprise sequences or portions of sequences useful in the manipulation of DNA molecules (restriction sites, transposition sites, sequencing primers, etc.). The primers of the invention may be used in nucleic acid synthesis and preferably are used for amplification (*e.g.*, PCR) of nucleic acid molecules. When the primers of the invention include target- or gene-specific sequences (any sequence contained within the target to be synthesized or amplified including translation signals, gene sequences, stop codons, transcriptional signals (*e.g.*, promoters) and the like), amplification or synthesis of target sequences or genes may be accomplished. Thus, the invention relates to synthesis of a nucleic acid molecules comprising mixing one or more primers of the invention with a nucleic acid

template, and incubating said mixture under conditions sufficient to make a first nucleic acid molecule complementary to all or a portion of said template. Thus, the invention relates specifically to a method of synthesizing a nucleic acid molecule comprising:

- (a) mixing a nucleic acid template with a polypeptide having polymerase activity and one or more primers comprising one or more recombination sites or portions thereof; and
- (b) incubating said mixture under conditions sufficient to synthesize a first nucleic acid molecule complementary to all or a portion of said template and which preferably comprises one or more recombination sites or portions thereof.

Such method of the invention may further comprise incubating said first synthesized nucleic acid molecule under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion of said first nucleic acid molecule. Such synthesis may provide for a first nucleic acid molecule having a recombination site or portion thereof at one or both of its termini.

In a preferred aspect, for the synthesis of the nucleic acid molecules, at least two primers are used wherein each primer comprises a homologous sequence at its terminus and/or within internal sequences of each primer (which may have a homology length of about 2 to about 500 bases, preferably about 3 to about 100 bases, about 4 to about 50 bases, about 5 to about 25 bases and most preferably about 6 to about 18 base overlap). In a preferred aspect, the first such primer comprises at least one target-specific sequence and at least one recombination site or portion thereof while the second primer comprises at least one recombination site or portion thereof. Preferably, the homologous regions between the first and second primers comprise at least a portion of the recombination site. In another aspect, the homologous regions between the first and second primers may comprise one or more additional sequences, *e.g.*, expression signals, translational start motifs, or other sequences adding functionality to the desired nucleic acid sequence upon amplification. In practice, two pairs of primers prime synthesis or amplification of a nucleic acid molecule. In a preferred aspect, all or at least a portion of the synthesized or amplified nucleic acid molecule will be homologous

to all or a portion of the template and further comprises a recombination site or a portion thereof at at least one terminus and preferably both termini of the synthesized or amplified molecule. Such synthesized or amplified nucleic acid molecule may be double stranded or single stranded and may be used in the recombinational cloning methods of the invention. The homologous primers of the invention provide a substantial advantage in that one set of the primers may be standardized for any synthesis or amplification reaction. That is, the primers providing the recombination site sequences (without the target specific sequences) can be pre-made and readily available for use. This in practice allows the use of shorter custom made primers that contain the target specific sequence needed to synthesize or amplify the desired nucleic acid molecule. Thus, this provides reduced time and cost in preparing target specific primers (e.g., shorter primers containing the target specific sequences can be prepared and used in synthesis reactions). The standardized primers, on the other hand, may be produced in mass to reduce cost and can be readily provided (e.g., in kits or as a product) to facilitate synthesis of the desired nucleic acid molecules.

Thus, in one preferred aspect, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and
- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.

More specifically, the invention relates to a method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- 5 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template specific sequence (complementary to or capable of hybridizing to said templates) and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
- 10 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one and preferably both termini of said molecules.
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In a more preferred aspect, the invention relates to a method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- 20 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and one or more first primers comprising at least a portion of a recombination site and a template specific sequence (complementary to or capable of hybridizing to said template);
- 25 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one and preferably both termini of said molecules;
- 30 (c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or



complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

- (d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one and preferably both termini of said molecules.

The invention also relates to vectors comprising the nucleic acid molecules of the invention, to host cells comprising the vectors or nucleic acid molecules of the invention, to methods of producing polypeptides encoded by the nucleic acid molecules of the invention, and to polypeptides encoded by these nucleic acid molecules or produced by the methods of the invention, which may be fusion proteins. The invention also relates to antibodies that bind to one or more polypeptides of the invention or epitopes thereof, which may be monoclonal or polyclonal antibodies. The invention also relates to the use of these nucleic acid molecules, primers, vectors, polypeptides and antibodies in methods for recombinational cloning of nucleic acids, *in vitro* and *in vivo*, to provide chimeric DNA molecules that have particular characteristics and/or DNA segments.

The antibodies of the invention may have particular use to identify and/or purify peptides or proteins (including fusion proteins produced by the invention), and to identify and/or purify the nucleic acid molecules of the invention or portions thereof.

The methods for *in vitro* or *in vivo* recombinational cloning of nucleic acid molecule generally relate to recombination between at least a first nucleic acid molecule having at least one recombination site and a second nucleic acid molecule having at least one recombination site to provide a chimeric nucleic acid molecule. In one aspect, the methods relate to recombination between and first vector having at least one recombination site and a second vector having at least one recombination site to provide a chimeric vector. In another aspect, a nucleic acid molecule having at least one recombination site is combined with a vector having at least one recombination site to provide a chimeric vector. In a most preferred aspect, the nucleic acid molecules or vectors used in recombination

comprise two or more recombination sites. In a more specific embodiment of the invention, the recombination methods relate to a Destination Reaction (also referred to herein as an "LR reaction") in which recombination occurs between an Entry clone and a Destination Vector. Such a reaction transfers the nucleic acid molecule of interest from the Entry Clone into the Destination Vector to create an Expression Clone. The methods of the invention also specifically relate to an Entry or Gateward reaction (also referred to herein as a "BP reaction") in which an Expression Clone is recombined with a Donor vector to produce an Entry clone. In other aspects, the invention relates to methods to prepare Entry clones by combining an Entry vector with at least one nucleic acid molecule (e.g., gene or portion of a gene). The invention also relates to conversion of a desired vector into a Destination Vector by including one or more (preferably at least two) recombination sites in the vector of interest. In a more preferred aspect, a nucleic acid molecule (e.g., a cassette) having at least two recombination sites flanking a selectable marker (e.g., a toxic gene or a genetic element preventing the survival of a host cell containing that gene or element, and/or preventing replication, partition or heritability of a nucleic acid molecule (e.g., a vector or plasmid) comprising that gene or element) is added to the vector to make a Destination Vector of the invention.

Preferred vectors for use in the invention include prokaryotic vectors, eukaryotic vectors, or vectors which may shuttle between various prokaryotic and/or eukaryotic systems (e.g. shuttle vectors). Preferred prokaryotic vectors for use in the invention include but are not limited to vectors which may propagate and/or replicate in gram negative and/or gram positive bacteria, including bacteria of the genera *Escherichia*, *Salmonella*, *Proteus*, *Clostridium*, *Klebsiella*, *Bacillus*, *Streptomyces*, and *Pseudomonas* and preferably in the species *E. coli*. Eukaryotic vectors for use in the invention include vectors which propagate and/or replicate in yeast cells, plant cells, mammalian cells, (particularly human and mouse), fungal cells, insect cells, nematode cells, fish cells and the like. Particular vectors of interest include but are not limited to cloning vectors, sequencing vectors, expression vectors, fusion vectors, two-hybrid vectors, gene therapy vectors, phage display vectors, gene-targeting vectors, PACs, BACs, YACs, MACs, and

reverse two-hybrid vectors. Such vectors may be used in prokaryotic and/or eukaryotic systems depending on the particular vector.

In another aspect, the invention relates to kits which may be used in carrying out the methods of the invention, and more specifically relates to cloning or subcloning kits and kits for carrying out the LR Reaction (*e.g.*, making an Expression Clone), for carrying out the BP Reaction (*e.g.*, making an Entry Clone), and for making Entry Clone and Destination Vector molecules of the invention. Such kits may comprise a carrier or receptacle being compartmentalized to receive and hold therein any number of containers. Such containers may contain any number of components for carrying out the methods of the invention or combinations of such components. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins or auxiliary factors or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix), one or more reaction buffers, one or more nucleotides, one or more primers of the invention, one or more restriction enzymes, one or more ligases, one or more polypeptides having polymerase activity (*e.g.*, one or more reverse transcriptases or DNA polymerases), one or more proteinases (*e.g.*, proteinase K or other proteinases), one or more Destination Vector molecules, one or more Entry Clone molecules, one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3.1 host cells, such as *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells), instructions for using the kits of the invention (*e.g.*, to carry out the methods of the invention), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, particularly one or more nucleic acid molecules comprising a nucleotide sequence encoding the one or more recombination sites or portions thereof of the invention. Preferably, such nucleic acid molecules comprise at least two recombination sites which flank a selectable

marker (*e.g.*, a toxic gene and/or antibiotic resistance gene). In a preferred aspect, such nucleic acid molecules are in the form of a cassette (*e.g.*, a linear nucleic acid molecule comprising one or more and preferably two or more recombination sites or portions thereof).

5           Kits for inserting or adding recombination sites to nucleic acid molecules of interest may comprise one or more nucleases (preferably restriction endonucleases), one or more ligases, one or more topoisomerases, one or more polymerases, and one or more nucleic acid molecules or adapters comprising one or more recombination sites. Kits for integrating recombination sites into one or  
10           more nucleic acid molecules of interest may comprise one or more components (or combinations thereof) selected from the group consisting of one or more integration sequences comprising one or more recombination sites. Such integration sequences may comprise one or more transposons, integrating viruses, homologous recombination sequences, RNA molecules, one or more host cells  
15           and the like.

          Kits for making the Entry Clone molecules of the invention may comprise any or a number of components and the composition of such kits may vary depending on the specific method involved. Such methods may involve inserting the nucleic acid molecules of interest into an Entry or Donor Vector by the  
20           recombinational cloning methods of the invention, or using conventional molecular biology techniques (*e.g.*, restriction enzyme digestion and ligation). In a preferred aspect, the Entry Clone is made using nucleic acid amplification or synthesis products. Kits for synthesizing Entry Clone molecules from amplification or synthesis products may comprise one or more components (or combinations  
25           thereof) selected from the group consisting of one or more Donor Vectors (*e.g.*, one or more attP vectors including, but not limited to, pDONR201 (Figure 49), pDONR202 (Figure 50), pDONR203 (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (Figure 53), and the like), one or more polypeptides having polymerase activity (preferably DNA polymerases and most preferably thermostable DNA polymerases), one or more proteinases, one or more  
30           reaction buffers, one or more nucleotides, one or more primers comprising one or

more recombination sites or portions thereof, and instructions for making one or more Entry Clones.

Kits for making the Destination vectors of the invention may comprise any number of components and the compositions of such kits may vary depending on the specific method involved. Such methods may include the recombination methods of the invention or conventional molecular biology techniques (e.g., restriction endonuclease digestion and ligation). In a preferred aspect, the Destination vector is made by inserting a nucleic acid molecule comprising at least one recombination site (or portion thereof) of the invention (preferably a nucleic acid molecule comprising at least two recombination sites or portions thereof flanking a selectable marker) into a desired vector to convert the desired vector into a Destination vector of the invention. Such kits may comprise at least one component (or combinations thereof) selected from the group consisting of one or more restriction endonucleases, one or more ligases, one or more polymerases, one or more nucleotides, reaction buffers, one or more nucleic acid molecules comprising at least one recombination site or portion thereof (preferably at least one nucleic acid molecule comprising at least two recombination sites flanking at least one selectable marker, such as a cassette comprising at least one selectable marker such as antibiotic resistance genes and/or toxic genes), and instructions for making such Destination vectors.

The invention also relates to kits for using the antibodies of the invention in identification and/or isolation of peptides and proteins (which may be fusion proteins) produced by the nucleic acid molecules of the invention, and for identification and/or isolation of the nucleic acid molecules of the invention or portions thereof. Such kits may comprise one or more components (or combination thereof) selected from the group consisting of one or more antibodies of the invention, one or more detectable labels, one or more solid supports and the like.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, in light of the following drawings and description of the invention, and in light of the claims.



## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts one general method of the present invention, wherein the starting (parent) DNA molecules can be circular or linear. The goal is to exchange the new subcloning vector D for the original cloning vector B. It is desirable in one embodiment to select for AD and against all the other molecules, including the Cointegrate. The square and circle are sites of recombination: *e.g.*, *lox* (such as *loxP*) sites, *att* sites, *etc.* For example, segment D can contain expression signals, protein fusion domains, new drug markers, new origins of replication, or specialized functions for mapping or sequencing DNA. It should be noted that the cointegrate molecule contains Segment D (Destination vector) adjacent to segment A (Insert), thereby juxtaposing functional elements in D with the insert in A. Such molecules can be used directly in vitro (*e.g.*, if a promoter is positioned adjacent to a gene-for in vitro transcription/translation) or in vivo (following isolation in a cell capable of propagating *ccdB*-containing vectors) by selecting for the selection markers in Segments B+D. As one skilled in the art will recognize, this single step method has utility in certain envisioned applications of the invention.

Figure 2 is a more detailed depiction of the recombinational cloning system of the invention, referred to herein as the "GATEWAY™ Cloning System." This figure depicts the production of Expression Clones via a "Destination Reaction," which may also be referred to herein as an "LR Reaction." A *kan<sup>r</sup>* vector (referred to herein as an "Entry clone") containing a DNA molecule of interest (*e.g.*, a gene) localized between an *attL1* site and an *attL2* site is reacted with an *amp<sup>r</sup>* vector (referred to herein as a "Destination Vector") containing a toxic or "death" gene localized between an *attR1* site and an *attR2* site, in the presence of GATEWAY™ LR Clonase™ Enzyme Mix (a mixture of Int, IHF and Xis). After incubation at 25°C for about 60 minutes, the reaction yields an *amp<sup>r</sup>* Expression Clone containing the DNA molecule of interest localized between an *attB1* site and an *attB2* site, and a *kan<sup>r</sup>* byproduct molecule, as well as intermediates. The reaction mixture may then be transformed into host cells (*e.g.*, *E. coli*) and clones containing the nucleic acid molecule of interest may



be selected by plating the cells onto ampicillin-containing media and picking amp<sup>r</sup> colonies.

Figure 3 is a schematic depiction of the cloning of a nucleic acid molecule from an Entry clone into multiple types of Destination vectors, to produce a variety of Expression Clones. Recombination between a given Entry clone and different types of Destination vectors (not shown), via the LR Reaction depicted in Figure 2, produces multiple different Expression Clones for use in a variety of applications and host cell types.

Figure 4 is a detailed depiction of the production of Entry Clones via a "BP reaction," also referred to herein as an "Entry Reaction" or a "Gateway Reaction." In the example shown in this figure, an amp<sup>r</sup> expression vector containing a DNA molecule of interest (e.g., a gene) localized between an *attB1* site and an *attB2* site is reacted with a kan<sup>r</sup> Donor vector (e.g., an *attP* vector; here, GATEWAY™ pDONR201 (see Figure 49A-C)) containing a toxic or "death" gene localized between an *attP1* site and an *attP2* site, in the presence of GATEWAY™ BP Clonase™ Enzyme Mix (a mixture of Int and IHF). After incubation at 25°C for about 60 minutes, the reaction yields a kan<sup>r</sup> Entry clone containing the DNA molecule of interest localized between an *attL1* site and an *attL2* site, and an amp<sup>r</sup> by-product molecule. The Entry clone may then be transformed into host cells (e.g., *E. coli*) and clones containing the Entry clone (and therefore the nucleic acid molecule of interest) may be selected by plating the cells onto kanamycin-containing media and picking kan<sup>r</sup> colonies. Although this figure shows an example of use of a kan<sup>r</sup> Donor vector, it is also possible to use Donor vectors containing other selection markers, such as the gentamycin resistance or tetracycline resistance markers, as discussed herein.

Figure 5 is a more detailed schematic depiction of the LR ("Destination") reaction (Figure 5A) and the BP ("Entry" or "Gateway") reaction (Figure 5B) of the GATEWAY™ Cloning System, showing the reactants, products and byproducts of each reaction.

Figure 6 shows the sequences of the attB1 and attB2 sites flanking a gene of interest after subcloning into a Destination Vector to create an Expression Clone.

Figure 7 is a schematic depiction of four ways to make Entry Clones using the compositions and methods of the invention: 1. using restriction enzymes and ligase; 2. starting with a cDNA library prepared in an attL Entry Vector; 3. using an Expression Clone from a library prepared in an attB Expression Vector via the BxP reaction; and 4. recombinational cloning of PCR fragments with terminal attB sites, via the BxP reaction. Approaches 3 and 4 rely on recombination with a Donor vector (here, an attP vector such as pDONR201 (see Figure 49A-C), pDONR202 (see Figure 50A-C), pDONR203 (see Figure 51A-C), pDONR204 (see Figure 52A-C), pDONR205 (see Figure 53A-C), or pDONR206 (see Figure 54A-C), for example) that provides an Entry Clone carrying a selection marker such as kan<sup>r</sup>, gen<sup>r</sup>, tet<sup>r</sup>, or the like.

Figure 8 is a schematic depiction of cloning of a PCR product by a BxP (Entry or Gateward) reaction. A PCR product with 25 bp terminal attB sites (plus four Gs) is shown as a substrate for the BxP reaction. Recombination between the attB-PCR product of a gene and a Donor vector (which donates an Entry Vector that carries kan<sup>r</sup>) results in an Entry Clone of the PCR product.

Figure 9 is a listing of the nucleotide sequences of the recombination sites designated herein as attB1, attB2, attP1, attP2, attL1, attL2, attR1 and attR2. Sequences are written conventionally, from 5' to 3'.

Figures 10-20: The plasmid backbone for all the Entry Vectors depicted herein is the same, and is shown in Figure 10A for the Entry Vector pENTR1A. For other Entry Vectors shown in Figures 11-20, only the sequences shown in Figure "A" for each figure set (i.e., Figure 11A, Figure 12A, etc.) are different (within the attL1-attL2 cassettes) from those shown in Figure 10 -- the plasmid backbone is identical.

Figure 10 is a schematic depiction of the physical map and cloning sites (Figure 10A), and the nucleotide sequence (Figure 10B), of the Entry Vector pENTR1A.

**Figure 11** is a schematic depiction of the cloning sites (Figure 11A) and the nucleotide sequence (Figure 11B) of the Entry Vector pENTR2B.

**Figure 12** is a schematic depiction of the cloning sites (Figure 12A) and the nucleotide sequence (Figure 12B) of the Entry Vector pENTR3C.

5 **Figure 13** is a schematic depiction of the cloning sites (Figure 13A) and the nucleotide sequence (Figure 13B) of the Entry Vector pENTR4.

**Figure 14** is a schematic depiction of the cloning sites (Figure 14A) and the nucleotide sequence (Figure 14B) of the Entry Vector pENTR5.

10 **Figure 15** is a schematic depiction of the cloning sites (Figure 15A) and the nucleotide sequence (Figure 15B) of the Entry Vector pENTR6.

**Figure 16** is a schematic depiction of the cloning sites (Figure 16A) and the nucleotide sequence (Figure 16B) of the Entry Vector pENTR7.

**Figure 17** is a schematic depiction of the cloning sites (Figure 17A) and the nucleotide sequence (Figure 17B) of the Entry Vector pENTR8.

15 **Figure 18** is a schematic depiction of the cloning sites (Figure 18A) and the nucleotide sequence (Figure 18B) of the Entry Vector pENTR9.

**Figure 19** is a schematic depiction of the cloning sites (Figure 19A) and the nucleotide sequence (Figure 19B) of the Entry Vector pENTR10.

20 **Figure 20** is a schematic depiction of the cloning sites (Figure 20A) and the nucleotide sequence (Figure 20B) of the Entry Vector pENTR11.

25 **Figure 21** is a schematic depiction of the physical map and the Trc expression cassette (Figure 21A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 21B-D), of Destination Vector pDEST1. This vector may also be referred to as pTrc-DEST1.

30 **Figure 22** is a schematic depiction of the physical map and the His6 expression cassette (Figure 22A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 22B-D), of Destination Vector pDEST2. This vector may also be referred to as pHis6-DEST2.

**Figure 23** is a schematic depiction of the physical map and the GST expression cassette (Figure 23A) showing the promoter sequences at -35 and at -10 from the initiation codon, and the nucleotide sequence (Figure 23B-D), of Destination Vector pDEST3. This vector may also be referred to as pGST-DEST3.

**Figure 24** is a schematic depiction of the physical map and the His6-Trx expression cassette (Figure 24A) showing the promoter sequences at -35 and at -10 from the initiation codon and a TEV protease cleavage site, and the nucleotide sequence (Figure 24B-D), of Destination Vector pDEST4. This vector may also be referred to as pTrx-DEST4.

**Figure 25** is a schematic depiction of the attR1 and attR2 sites (Figure 25A), the physical map (Figure 25B), and the nucleotide sequence (Figure 25C-D), of Destination Vector pDEST5. This vector may also be referred to as pSPORT(+)-DEST5.

**Figure 26** is a schematic depiction of the attR1 and attR2 sites (Figure 26A), the physical map (Figure 26B), and the nucleotide sequence (Figure 26C-D), of Destination Vector pDEST6. This vector may also be referred to as pSPORT(-)-DEST6.

**Figure 27** is a schematic depiction of the attR1 site, CMV promoter, and the physical map (Figure 27A), and the nucleotide sequence (Figure 27B-C), of Destination Vector pDEST7. This vector may also be referred to as pCMV-DEST7.

**Figure 28** is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, and the physical map (Figure 28A), and the nucleotide sequence (Figure 28B-D), of Destination Vector pDEST8. This vector may also be referred to as pFastBac-DEST8.

**Figure 29** is a schematic depiction of the attR1 site, Semliki Forest Virus promoter, and the physical map (Figure 29A), and the nucleotide sequence (Figure 29B-E), of Destination Vector pDEST9. This vector may also be referred to as pSFV-DEST9.

**Figure 30** is a schematic depiction of the attR1 site, baculovirus polyhedrin promoter, His6 fusion domain, and the physical map (Figure 30A), and the nucleotide sequence (Figure 30B-D), of Destination Vector pDEST10. This vector may also be referred to as pFastBacHT-DEST10.

**Figure 31** is a schematic depiction of the attR1 cassette containing a tetracycline-regulated CMV promoter and the physical map (Figure 31A), and the nucleotide sequence (Figure 31B-D), of Destination Vector pDEST11. This vector may also be referred to as pTet-DEST11.

**Figure 32** is a schematic depiction of the attR1 site, the start of the mRNA of the CMV promoter, and the physical map (Figure 32A), and the nucleotide sequence (Figure 32B-D), of Destination Vector pDEST12.2. This vector may also be referred to as pCMVneo-DEST12, as pCMV-DEST12, or as pDEST12.

**Figure 33** is a schematic depiction of the attR1 site, the  $\lambda P_L$  promoter, and the physical map (Figure 33A), and the nucleotide sequence (Figure 33B-C), of Destination Vector pDEST13. This vector may also be referred to as p $\lambda P_L$ -DEST13.

**Figure 34** is a schematic depiction of the attR1 site, the T7 promoter, and the physical map (Figure 34A), and the nucleotide sequence (Figure 34B-D), of Destination Vector pDEST14. This vector may also be referred to as pT7-DEST14.

**Figure 35** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 35A), and the nucleotide sequence (Figure 35B-D), of Destination Vector pDEST15. This vector may also be referred to as pT7 GST-DEST15.

**Figure 36** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal thioredoxin fusion sequence, and the physical map (Figure 36A), and the nucleotide sequence (Figure 36B-D), of Destination Vector pDEST16. This vector may also be referred to as pT7 Trx-DEST16.

**Figure 37** is a schematic depiction of the attR1 site, the T7 promoter, and the N-terminal His6 fusion sequence, and the physical map (Figure 37A), and the

nucleotide sequence (Figure 37B-D), of Destination Vector pDEST17. This vector may also be referred to as pT7 His-DEST17.

Figure 38 is a schematic depiction of the attR1 site and the p10 baculovirus promoter, and the physical map (Figure 38A), and the nucleotide sequence (Figure 38B-D), of Destination Vector pDEST18. This vector may also be referred to as pFBp10-DEST18.

Figure 39 is a schematic depiction of the attR1 site, and the 39k baculovirus promoter, and the physical map (Figure 39A), and the nucleotide sequence (Figure 39B-D), of Destination Vector pDEST19. This vector may also be referred to as pFB39k-DEST19.

Figure 40 is a schematic depiction of the attR1 site, the *polh* baculovirus promoter, and the N-terminal GST fusion sequence, and the physical map (Figure 40A), and the nucleotide sequence (Figure 40B-D), of Destination Vector pDEST20. This vector may also be referred to as pFB GST-DEST20.

Figure 41 is a schematic depiction of a 2-hybrid vector with a DNA-binding domain, the attR1 site, and the ADH promoter, and the physical map (Figure 41A), and the nucleotide sequence (Figure 41B-E), of Destination Vector pDEST21. This vector may also be referred to as pDB Leu-DEST21.

Figure 42 is a schematic depiction of a 2-hybrid vector with an activation domain, the attR1 site, and the ADH promoter, and the physical map (Figure 42A), and the nucleotide sequence (Figure 42B-D), of Destination Vector pDEST22. This vector may also be referred to as pPC86-DEST22.

Figure 43 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal His6 fusion sequence, and the physical map (Figure 43A), and the nucleotide sequence (Figure 43B-D), of Destination Vector pDEST23. This vector may also be referred to as pC-term-His6-DEST23.

Figure 44 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal GST fusion sequence, and the physical map (Figure 44A), and the nucleotide sequence (Figure 44B-D), of Destination Vector pDEST24. This vector may also be referred to as pC-term-GST-DEST24.



Figure 45 is a schematic depiction of the attR1 and attR2 sites, the T7 promoter, and the C-terminal thioredoxin fusion sequence, and the physical map (Figure 45A), and the nucleotide sequence (Figure 45B-D), of Destination Vector pDEST25. This vector may also be referred to as pC-term-Trx-DEST25.

5 Figure 46 is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal His6 fusion sequence, and the physical map (Figure 46A), and the nucleotide sequence (Figure 46B-D), of Destination Vector pDEST26. This vector may also be referred to as pCMV-SPneo-His-DEST26.

10 Figure 47 is a schematic depiction of the attR1 site, the CMV promoter, and an N-terminal GST fusion sequence, and the physical map (Figure 47A), and the nucleotide sequence (Figure 47B-D), of Destination Vector pDEST27. This vector may also be referred to as pCMV-Spneo-GST-DEST27.

15 Figure 48 is a depiction of the physical map (Figure 48A), the cloning sites (Figure 48B), and the nucleotide sequence (Figure 48C-D), for the attB cloning vector plasmid pEXP501. This vector may also be referred to equivalently herein as pCMV-SPORT6, pCMVSPORT6, and pCMVSpport6.

20 Figure 49 is a depiction of the physical map (Figure 49A), and the nucleotide sequence (Figure 49B-C), for the Donor plasmid pDONR201 which donates a kanamycin-resistant vector in the BP Reaction. This vector may also be referred to as pAttPkanr Donor Plasmid, or as pAttPkan Donor Plasmid

Figure 50 is a depiction of the physical map (Figure 50A), and the nucleotide sequence (Figure 50B-C), for the Donor plasmid pDONR202 which donates a kanamycin-resistant vector in the BP Reaction.

25 Figure 51 is a depiction of the physical map (Figure 51A), and the nucleotide sequence (Figure 51B-C), for the Donor plasmid pDONR203 which donates a kanamycin-resistant vector in the BP Reaction.

Figure 52 is a depiction of the physical map (Figure 52A), and the nucleotide sequence (Figure 52B-C), for the Donor plasmid pDONR204 which donates a kanamycin-resistant vector in the BP Reaction.

Figure 53 is a depiction of the physical map (Figure 53A), and the nucleotide sequence (Figure 53B-C), for the Donor plasmid pDONR205 which donates a tetracycline-resistant vector in the BP Reaction.

5 Figure 54 is a depiction of the physical map (Figure 54A), and the nucleotide sequence (Figure 54B-C), for the Donor plasmid pDONR206 which donates a gentamycin-resistant vector in the BP Reaction. This vector may also be referred to as pENTR22 attP Donor Plasmid, pAttPGenr Donor Plasmid, or pAttPgnt Donor Plasmid.

10 Figure 55 depicts the attB1 site, and the physical map, of an Entry Clone (pENTR7) of CAT subcloned into the Destination Vector pDEST2 (Figure 22).

Figure 56 depicts the DNA components of Reaction B of the one-tube BxP reaction described in Example 16, pEZC7102 and attB-tet-PCR.

Figure 57 is a physical map of the desired product of Reaction B of the one-tube BxP reaction described in Example 16, tetx7102.

15 Figure 58 is a physical map of the Destination Vector pEZC8402.

Figure 59 is a physical map of the expected tet<sup>r</sup> subclone product, tetx8402, resulting from the LxR Reaction with tetx7102 (Figure 57) plus pEZC8402 (Figure 58).

20 Figure 60 is a schematic depiction of the bacteriophage lambda recombination pathways in *E. coli*.

25 Figure 61 is a schematic depiction of the DNA molecules participating in the LR Reaction. Two different co-integrates form during the LR Reaction (only one of which is shown here), depending on whether attL1 and attR1 or attL2 and attR2 are first to recombine. In one aspect, the invention provides directional cloning of a nucleic acid molecule of interest, since the recombination sites react with specificity (attL1 reacts with attR1; attL2 with attR2; attB1 with attP1; and attB2 with attP2). Thus, positioning of the sites allows construction of desired vectors having recombined fragments in the desired orientation.

30 Figure 62 is a depiction of native and fusion protein expression using the recombinational cloning methods and compositions of the invention. In the upper figure depicting native protein expression, all of the translational start signals are

included between the attB1 and attB2 sites; therefore, these signals must be present in the starting Entry Clone. The lower figure depicts fusion protein expression (here showing expression with both N-terminal and C-terminal fusion tags so that ribosomes read through attB1 and attB2 to create the fusion protein). Unlike native protein expression vectors, N-terminal fusion vectors have their translational start signals upstream of the attB1 site.

Figure 63 is a schematic depiction of three GATEWAY™ Cloning System cassettes. Three blunt-ended cassettes are depicted which convert standard expression vectors to Destination Vectors. Each of the depicted cassettes provides amino-terminal fusions in one of three possible reading frames, and each has a distinctive restriction cleavage site as shown.

Figure 64 shows the physical maps of plasmids containing three attR reading frame cassettes, pEZC15101 (reading frame A; Figure 64A), pEZC15102 (reading frame B; Figure 64B), and pEZC15103 (reading frame C; Figure 64C).

Figure 65 depicts the attB primers used for amplifying the tet<sup>r</sup> and amp<sup>r</sup> genes from pBR322 by the cloning methods of the invention.

Figure 66 is a table listing the results of recombinational cloning of the tet<sup>r</sup> and amp<sup>r</sup> PCR products made using the primers shown in Figure 65.

Figure 67 is a graph showing the effect of the number of guanines (G's) contained on the 5' end of the PCR primers on the cloning efficiency of PCR products. It is noted, however, that other nucleotides besides guanine (including A, T, C, U or combinations thereof) may be used as 5' extensions on the PCR primers to enhance cloning efficiency of PCR products.

Figure 68 is a graph showing a titration of various amounts of attP and attB reactants in the BxP reaction, and the effects on cloning efficiency of PCR products.

Figure 69 is a series of graphs showing the effects of various weights (Figure 69A) or moles (Figure 69B) of a 256 bp PCR product on formation of colonies, and on efficiency of cloning of the 256 bp PCR product into a Donor Vector (Figure 69C).

Figure 70 is a series of graphs showing the effects of various weights (Figure 70A) or moles (Figure 70B) of a 1 kb PCR product on formation of colonies, and on efficiency of cloning of the 1 kb PCR product into a Donor Vector (Figure 70C).

5 Figure 71 is a series of graphs showing the effects of various weights (Figure 71A) or moles (Figure 71B) of a 1.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 1.4 kb PCR product into a Donor Vector (Figure 71C).

10 Figure 72 is a series of graphs showing the effects of various weights (Figure 72A) or moles (Figure 72B) of a 3.4 kb PCR product on formation of colonies, and on efficiency of cloning of the 3.4 kb PCR product into a Donor Vector (Figure 72C).

15 Figure 73 is a series of graphs showing the effects of various weights (Figure 73A) or moles (Figure 73B) of a 4.6 kb PCR product on formation of colonies, and on efficiency of cloning of the 4.6 kb PCR product into a Donor Vector (Figure 73C).

Figure 74 is photograph of an ethidium bromide-stained gel of a titration of a 6.9 kb PCR product in a BxP reaction.

20 Figure 75 is a graph showing the effects of various amounts of a 10.1 kb PCR product on formation of colonies upon cloning of the 10.1 kb PCR product into a Donor Vector.

Figure 76 is photograph of an ethidium bromide-stained gel of a titration of a 10.1 kb PCR product in a BxP reaction.

25 Figure 77 is a table summarizing the results of the PCR product cloning efficiency experiments depicted in Figures 69-74, for PCR fragments ranging in size from 0.256 kb to 6.9 kb.

30 Figure 78 is a depiction of the sequences at the ends of attR Cassettes. Sequences contributed by the  $Cm^r$ -ccdB cassette are shown, including the outer ends of the flanking attR sites (boxed). The staggered cleavage sites for Int are indicated in the boxed regions. Following recombination with an Entry Clone, only the outer sequences in attR sites contribute to the resulting attB sites in the

Expression Clone. The underlined sequences at both ends dictate the different reading frames (reading frames A, B, or C, with two alternative reading frame C cassettes depicted) for fusion proteins.

Figure 79 is a depiction of several different attR cassettes (in reading frames A, B, or C) which may provide fusion codons at the amino-terminus of the encoded protein.

Figure 80 illustrates the single-cutting restriction sites in an attR reading frame A cassette of the invention.

Figure 81 illustrates the single-cutting restriction sites in an attR reading frame B cassette of the invention.

Figure 82 illustrates the single-cutting restriction sites in two alternative attR reading frame C cassettes of the invention (Figures 82A and 82B) depicted in Figure 78.

Figure 83 shows the physical map (Figure 83A), and the nucleotide sequence (Figure 83B-C), for an attR reading frame C parent plasmid prfC Parent III, which contains an attR reading frame C cassette of the invention (alternative A in Figures 78 and 82).

Figure 84 is a physical map of plasmid pEYC1301.

Figure 85 is a physical map of plasmid pEYC1313.

Figure 86 is a physical map of plasmid pEYC14032.

Figure 87 is a physical map of plasmid pMAB58.

Figure 88 is a physical map of plasmid pMAB62.

Figure 89 is a depiction of a synthesis reaction using two pairs of homologous primers of the invention.

Figure 90 is a schematic depiction of the physical map (Figure 90A), and the nucleotide sequence (Figure 90B-D), of Destination Vector pDEST28.

Figure 91 is a schematic depiction of the physical map (Figure 91A), and the nucleotide sequence (Figure 91B-D), of Destination Vector pDEST29.

Figure 92 is a schematic depiction of the physical map (Figure 92A), and the nucleotide sequence (Figure 92B-D), of Destination Vector pDEST30.

Figure 93 is a schematic depiction of the physical map (Figure 93A), and the nucleotide sequence (Figure 93B-D), of Destination Vector pDEST31.

Figure 94 is a schematic depiction of the physical map (Figure 94A), and the nucleotide sequence (Figure 94B-E), of Destination Vector pDEST32.

5 Figure 95 is a schematic depiction of the physical map (Figure 95A), and the nucleotide sequence (Figure 95B-D), of Destination Vector pDEST33.

Figure 96 is a schematic depiction of the physical map (Figure 96A), and the nucleotide sequence (Figure 96B-D), of Destination Vector pDEST34.

10 Figure 97 is a depiction of the physical map (Figure 97A), and the nucleotide sequence (Figure 97B-C), for the Donor plasmid pDONR207 which donates a gentamycin-resistant vector in the BP Reaction.

Figure 98 is a schematic depiction of the physical map (Figure 98A), and the nucleotide sequence (Figure 98B-D), of the 2-hybrid vector pMAB85.

15 Figure 99 is a schematic depiction of the physical map (Figure 99A), and the nucleotide sequence (Figure 99B-D), of the 2-hybrid vector pMAB86.

## DETAILED DESCRIPTION OF THE INVENTION

### 20 Definitions

In the description that follows, a number of terms used in recombinant DNA technology are utilized extensively. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

25 **Byproduct:** is a daughter molecule (a new clone produced after the second recombination event during the recombinational cloning process) lacking the segment which is desired to be cloned or subcloned.

30 **Cointegrate:** is at least one recombination intermediate nucleic acid molecule of the present invention that contains both parental (starting) molecules. It will usually be linear. In some embodiments it can be circular. RNA and polypeptides may be expressed from cointegrates using an appropriate host cell strain, for example *E. coli* DB3.1 (particularly *E. coli* LIBRARY EFFICIENCY®).



DB3.1™ Competent Cells), and selecting for both selection markers found on the cointegrate molecule.

**Host:** is any prokaryotic or eukaryotic organism that can be a recipient of the recombinational cloning Product, vector, or nucleic acid molecule of the invention. A "host," as the term is used herein, includes prokaryotic or eukaryotic organisms that can be genetically engineered. For examples of such hosts, see Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

**Insert or Inserts:** include the desired nucleic acid segment or a population of nucleic acid segments (segment *A* of Figure 1) which may be manipulated by the methods of the present invention. Thus, the terms Insert(s) are meant to include a particular nucleic acid (preferably DNA) segment or a population of segments. Such Insert(s) can comprise one or more nucleic acid molecules.

**Insert Donor:** is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the Insert. The Insert Donor molecule comprises the Insert flanked on both sides with recombination sites. The Insert Donor can be linear or circular. In one embodiment of the invention, the Insert Donor is a circular DNA molecule and further comprises a cloning vector sequence outside of the recombination signals (see Figure 1). When a population of Inserts or population of nucleic acid segments are used to make the Insert Donor, a population of Insert Donors results and may be used in accordance with the invention. Examples of such Insert Donor molecules are GATEWAY™ Entry Vectors, which include but are not limited to those Entry Vectors depicted in Figures 10-20, as well as other vectors comprising a gene of interest flanked by one or more *attL* sites (e.g., *attL1*, *attL2*, etc.), or by one or more *attB* sites (e.g., *attB1*, *attB2*, etc.) for the production of library clones.

**Product:** is one of the desired daughter molecules comprising the *A* and *D* sequences which is produced after the second recombination event during the recombinational cloning process (see Figure 1). The Product contains the nucleic acid which was to be cloned or subcloned. In accordance with the invention, when a population of Insert Donors are used, the resulting population of Product

molecules will contain all or a portion of the population of Inserts of the Insert Donors and preferably will contain a representative population of the original molecules of the Insert Donors.

**Promoter:** is a DNA sequence generally described as the 5'-region of a gene, located proximal to the start codon. The transcription of an adjacent DNA segment is initiated at the promoter region. A repressible promoter's rate of transcription decreases in response to a repressing agent. An inducible promoter's rate of transcription increases in response to an inducing agent. A constitutive promoter's rate of transcription is not specifically regulated, though it can vary under the influence of general metabolic conditions.

**Recognition sequence:** Recognition sequences are particular sequences which a protein, chemical compound, DNA, or RNA molecule (e.g., restriction endonuclease, a modification methylase, or a recombinase) recognizes and binds. In the present invention, a recognition sequence will usually refer to a recombination site. For example, the recognition sequence for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Current Opinion in Biotechnology* 5:521-527 (1994). Other examples of recognition sequences are the *attB*, *attP*, *attL*, and *attR* sequences which are recognized by the recombinase enzyme  $\lambda$  Integrase. *attB* is an approximately 25 base pair sequence containing two 9 base pair core-type Int binding sites and a 7 base pair overlap region. *attP* is an approximately 240 base pair sequence containing core-type Int binding sites and arm-type Int binding sites as well as sites for auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993). Such sites may also be engineered according to the present invention to enhance production of products in the methods of the invention. When such engineered sites lack the P1 or H1 domains to make the recombination reactions irreversible (e.g., *attR* or *attP*), such sites may be designated *attR'* or *attP'* to show that the domains of these sites have been modified in some way.

**Recombination proteins:** include excisive or integrative proteins, enzymes, co-factors or associated proteins that are involved in recombination reactions involving one or more recombination sites, which may be wild-type proteins (See Landy, *Current Opinion in Biotechnology* 3:699-707 (1993)), or mutants, derivatives (e.g., fusion proteins containing the recombination protein sequences or fragments thereof), fragments, and variants thereof.

**Recombination site:** is a recognition sequence on a DNA molecule participating in an integration/recombination reaction by the recombinational cloning methods of the invention. Recombination sites are discrete sections or segments of DNA on the participating nucleic acid molecules that are recognized and bound by a site-specific recombination protein during the initial stages of integration or recombination. For example, the recombination site for Cre recombinase is *loxP* which is a 34 base pair sequence comprised of two 13 base pair inverted repeats (serving as the recombinase binding sites) flanking an 8 base pair core sequence. See Figure 1 of Sauer, B., *Curr. Opin. Biotech.* 5:521-527 (1994). Other examples of recognition sequences include the *attB*, *attP*, *attL*, and *attR* sequences described herein, and mutants, fragments, variants and derivatives thereof, which are recognized by the recombination protein  $\lambda$  Int and by the auxiliary proteins integration host factor (IHF), FIS and excisionase (Xis). See Landy, *Curr. Opin. Biotech.* 3:699-707 (1993).

**Recombinational Cloning:** is a method described herein, whereby segments of nucleic acid molecules or populations of such molecules are exchanged, inserted, replaced, substituted or modified, *in vitro* or *in vivo*. By "*in vitro*" and "*in vivo*" herein is meant recombinational cloning that is carried out outside of host cells (e.g., in cell-free systems) or inside of host cells (e.g., using recombination proteins expressed by host cells), respectively.

**Repression cassette:** is a nucleic acid segment that contains a repressor or a Selectable marker present in the subcloning vector.

**Selectable marker:** is a DNA segment that allows one to select for or against a molecule (e.g., a replicon) or a cell that contains it, often under particular conditions. These markers can encode an activity, such as, but not limited to,

production of RNA, peptide, or protein, or can provide a binding site for RNA, peptides, proteins, inorganic and organic compounds or compositions and the like. Examples of Selectable markers include but are not limited to: (1) DNA segments that encode products which provide resistance against otherwise toxic compounds (e.g., antibiotics); (2) DNA segments that encode products which are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); (3) DNA segments that encode products which suppress the activity of a gene product; (4) DNA segments that encode products which can be readily identified (e.g., phenotypic markers such as  $\beta$ -galactosidase, green fluorescent protein (GFP), and cell surface proteins); (5) DNA segments that bind products which are otherwise detrimental to cell survival and/or function; (6) DNA segments that otherwise inhibit the activity of any of the DNA segments described in Nos. 1-5 above (e.g., antisense oligonucleotides); (7) DNA segments that bind products that modify a substrate (e.g. restriction endonucleases); (8) DNA segments that can be used to isolate or identify a desired molecule (e.g. specific protein binding sites); (9) DNA segments that encode a specific nucleotide sequence which can be otherwise non-functional (e.g., for PCR amplification of subpopulations of molecules); (10) DNA segments, which when absent, directly or indirectly confer resistance or sensitivity to particular compounds; (11) DNA segments that encode products which are toxic in recipient cells; (12) DNA segments that inhibit replication, partition or heritability of nucleic acid molecules that contain them; and/or (13) DNA segments that encode conditional replication functions, e.g., replication in certain hosts or host cell strains or under certain environmental conditions (e.g., temperature, nutritional conditions, etc.).

**Selection scheme:** is any method which allows selection, enrichment, or identification of a desired Product or Product(s) from a mixture containing an Entry Clone or Vector, a Destination Vector, a Donor Vector, an Expression Clone or Vector, any intermediates (e.g. a Cointegrate or a replicon), and/or Byproducts. The selection schemes of one preferred embodiment have at least two components that are either linked or unlinked during recombinational cloning. One component is a Selectable marker. The other component controls the expression *in vitro* or *in vivo* of the Selectable marker, or survival of the cell (or

the nucleic acid molecule, *e.g.*, a replicon) harboring the plasmid carrying the Selectable marker. Generally, this controlling element will be a repressor or inducer of the Selectable marker, but other means for controlling expression or activity of the Selectable marker can be used. Whether a repressor or activator is used will depend on whether the marker is for a positive or negative selection, and the exact arrangement of the various DNA segments, as will be readily apparent to those skilled in the art. A preferred requirement is that the selection scheme results in selection of or enrichment for only one or more desired Products. As defined herein, selecting for a DNA molecule includes (a) selecting or enriching for the presence of the desired DNA molecule, and (b) selecting or enriching against the presence of DNA molecules that are not the desired DNA molecule.

In one embodiment, the selection schemes (which can be carried out in reverse) will take one of three forms, which will be discussed in terms of Figure 1. The first, exemplified herein with a Selectable marker and a repressor therefore, selects for molecules having segment *D* and lacking segment *C*. The second selects against molecules having segment *C* and for molecules having segment *D*. Possible embodiments of the second form would have a DNA segment carrying a gene toxic to cells into which the *in vitro* reaction products are to be introduced. A toxic gene can be a DNA that is expressed as a toxic gene product (a toxic protein or RNA), or can be toxic in and of itself. (In the latter case, the toxic gene is understood to carry its classical definition of "heritable trait".)

Examples of such toxic gene products are well known in the art, and include, but are not limited to, restriction endonucleases (*e.g.*, *DpnI*), apoptosis-related genes (*e.g.* ASK1 or members of the *bcl-2/ced-9* family), retroviral genes including those of the human immunodeficiency virus (HIV), defensins such as NP-1, inverted repeats or paired palindromic DNA sequences, bacteriophage lytic genes such as those from  $\Phi$ X174 or bacteriophage T4; antibiotic sensitivity genes such as *rpsL*, antimicrobial sensitivity genes such as *pheS*, plasmid killer genes, eukaryotic transcriptional vector genes that produce a gene product toxic to bacteria, such as GATA-1, and genes that kill hosts in the absence of a suppressing function, *e.g.*, *kicB*, *ccaB*,  $\Phi$ X174 *E* (Liu, Q. *et al.*, *Curr. Biol.*



8:1300-1309 (1998)), and other genes that negatively affect replicon stability and/or replication. A toxic gene can alternatively be selectable *in vitro*, e.g., a restriction site.

Many genes coding for restriction endonucleases operably linked to inducible promoters are known, and may be used in the present invention. See, e.g. U.S. Patent Nos. 4,960,707 (*DpnI* and *DpnII*); 5,000,333, 5,082,784 and 5,192,675 (*KpnI*); 5,147,800 (*NgoAIII* and *NgoAI*); 5,179,015 (*FspI* and *HaeIII*); 5,200,333 (*HaeII* and *TaqI*); 5,248,605 (*HpaII*); 5,312,746 (*ClaI*); 5,231,021 and 5,304,480 (*XhoI* and *XhoII*); 5,334,526 (*AluI*); 5,470,740 (*NsiI*); 5,534,428 (*SstII/SacI*); 5,202,248 (*NcoI*); 5,139,942 (*NdeI*); and 5,098,839 (*PacI*). See also Wilson, G.G., *Nucl. Acids Res.* 19:2539-2566 (1991); and Lunnen, K.D., *et al.*, *Gene* 74:25-32 (1988).

In the second form, segment *D* carries a Selectable marker. The toxic gene would eliminate transformants harboring the Vector Donor, Cointegrate, and Byproduct molecules, while the Selectable marker can be used to select for cells containing the Product and against cells harboring only the Insert Donor.

The third form selects for cells that have both segments *A* and *D* in *cis* on the same molecule, but not for cells that have both segments in *trans* on different molecules. This could be embodied by a Selectable marker that is split into two inactive fragments, one each on segments *A* and *D*.

The fragments are so arranged relative to the recombination sites that when the segments are brought together by the recombination event, they reconstitute a functional Selectable marker. For example, the recombinational event can link a promoter with a structural nucleic acid molecule (e.g., a gene), can link two fragments of a structural nucleic acid molecule, or can link nucleic acid molecules that encode a heterodimeric gene product needed for survival, or can link portions of a replicon.

**Site-specific recombinase:** is a type of recombinase which typically has at least the following four activities (or combinations thereof): (1) recognition of one or two specific nucleic acid sequences; (2) cleavage of said sequence or sequences; (3) topoisomerase activity involved in strand exchange; and (4) ligase



activity to reseal the cleaved strands of nucleic acid. See Sauer, B., *Current Opinions in Biotechnology* 5:521-527 (1994). Conservative site-specific recombination is distinguished from homologous recombination and transposition by a high degree of sequence specificity for both partners. The strand exchange mechanism involves the cleavage and rejoining of specific DNA sequences in the absence of DNA synthesis (Landy, A. (1989) *Ann. Rev. Biochem.* 58:913-949).

**Subcloning vector:** is a cloning vector comprising a circular or linear nucleic acid molecule which includes preferably an appropriate replicon. In the present invention, the subcloning vector (segment *D* in Figure 1) can also contain functional and/or regulatory elements that are desired to be incorporated into the final product to act upon or with the cloned DNA Insert (segment *A* in Figure 1). The subcloning vector can also contain a Selectable marker (preferably DNA).

**Vector:** is a nucleic acid molecule (preferably DNA) that provides a useful biological or biochemical property to an Insert. Examples include plasmids, phages, autonomously replicating sequences (ARS), centromeres, and other sequences which are able to replicate or be replicated *in vitro* or in a host cell, or to convey a desired nucleic acid segment to a desired location within a host cell. A Vector can have one or more restriction endonuclease recognition sites at which the sequences can be cut in a determinable fashion without loss of an essential biological function of the vector, and into which a nucleic acid fragment can be spliced in order to bring about its replication and cloning. Vectors can further provide primer sites, *e.g.*, for PCR, transcriptional and/or translational initiation and/or regulation sites, recombinational signals, replicons, Selectable markers, *etc.* Clearly, methods of inserting a desired nucleic acid fragment which do not require the use of homologous recombination, transpositions or restriction enzymes (such as, but not limited to, UDG cloning of PCR fragments (U.S. Patent No. 5,334,575, entirely incorporated herein by reference), T:A cloning, and the like) can also be applied to clone a fragment into a cloning vector to be used according to the present invention. The cloning vector can further contain one or more selectable markers suitable for use in the identification of cells transformed with the cloning vector.

**Vector Donor:** is one of the two parental nucleic acid molecules (e.g. RNA or DNA) of the present invention which carries the DNA segments comprising the DNA vector which is to become part of the desired Product. The Vector Donor comprises a subcloning vector *D* (or it can be called the cloning vector if the Insert Donor does not already contain a cloning vector (e.g., for PCR fragments containing *attB* sites; see below)) and a segment *C* flanked by recombination sites (see Figure 1). Segments *C* and/or *D* can contain elements that contribute to selection for the desired Product daughter molecule, as described above for selection schemes. The recombination signals can be the same or different, and can be acted upon by the same or different recombinases. In addition, the Vector Donor can be linear or circular. Examples of such Vector Donor molecules include GATEWAY™ Destination Vectors, which include but are not limited to those Destination Vectors depicted in Figures 21-47 and 90-96.

**Primer:** refers to a single stranded or double stranded oligonucleotide that is extended by covalent bonding of nucleotide monomers during amplification or polymerization of a nucleic acid molecule (e.g. a DNA molecule). In a preferred aspect, a primer comprises one or more recombination sites or portions of such recombination sites. Portions of recombination sites comprise at least 2 bases (or basepairs, abbreviated herein as "bp"), at least 5-200 bases, at least 10-100 bases, at least 15-75 bases, at least 15-50 bases, at least 15-25 bases, or at least 16-25 bases, of the recombination sites of interest, as described in further detail below and in the Examples. When using portions of recombination sites, the missing portion of the recombination site may be provided as a template by the newly synthesized nucleic acid molecule. Such recombination sites may be located within and/or at one or both termini of the primer. Preferably, additional sequences are added to the primer adjacent to the recombination site(s) to enhance or improve recombination and/or to stabilize the recombination site during recombination. Such stabilization sequences may be any sequences (preferably G/C rich sequences) of any length. Preferably, such sequences range in size from 1 to about 1000 bases, 1 to about 500 bases, and 1 to about 100 bases, 1 to about 60 bases, 1 to about 25, 1 to about 10, 2 to about 10 and preferably about 4 bases.

Preferably, such sequences are greater than 1 base in length and preferably greater than 2 bases in length.

**Template:** refers to double stranded or single stranded nucleic acid molecules which are to be amplified, synthesized or sequenced. In the case of double stranded molecules, denaturation of its strands to form a first and a second strand is preferably performed before these molecules will be amplified, synthesized or sequenced, or the double stranded molecule may be used directly as a template. For single stranded templates, a primer complementary to a portion of the template is hybridized under appropriate conditions and one or more polypeptides having polymerase activity (e.g. DNA polymerases and/or reverse transcriptases) may then synthesize a nucleic acid molecule complementary to all or a portion of said template. Alternatively, for double stranded templates, one or more promoters may be used in combination with one or more polymerases to make nucleic acid molecules complementary to all or a portion of the template. The newly synthesized molecules, according to the invention, may be equal or shorter in length than the original template. Additionally, a population of nucleic acid templates may be used during synthesis or amplification to produce a population of nucleic acid molecules typically representative of the original template population.

**Adapter:** is an oligonucleotide or nucleic acid fragment or segment (preferably DNA) which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear Insert Donor molecule as well as other nucleic acid molecules described herein. When using portions of recombination sites, the missing portion may be provided by the Insert Donor molecule. Such adapters may be added at any location within a circular or linear molecule, although the adapters are preferably added at or near one or both termini of a linear molecule. Preferably, adapters are positioned to be located on both sides (flanking) a particular nucleic acid molecule of interest. In accordance with the invention, adapters may be added to nucleic acid molecules of interest by standard recombinant techniques (e.g. restriction digest and ligation). For example, adapters may be added to a circular molecule by first digesting the molecule with

an appropriate restriction enzyme, adding the adapter at the cleavage site and reforming the circular molecule which contains the adapter(s) at the site of cleavage. In other aspects, adapters may be added by homologous recombination, by integration of RNA molecules, and the like. Alternatively, adapters may be ligated directly to one or more and preferably both termini of a linear molecule thereby resulting in linear molecule(s) having adapters at one or both termini. In one aspect of the invention, adapters may be added to a population of linear molecules, (e.g. a cDNA library or genomic DNA which has been cleaved or digested) to form a population of linear molecules containing adapters at one and preferably both termini of all or substantial portion of said population.

**Adapter-Primer:** is primer molecule which comprises one or more recombination sites (or portions of such recombination sites) which in accordance with the invention can be added to a circular or linear nucleic acid molecule described herein. When using portions of recombination sites, the missing portion may be provided by a nucleic acid molecule (e.g., an adapter) of the invention. Such adapter-primers may be added at any location within a circular or linear molecule, although the adapter-primers are preferably added at or near one or both termini of a linear molecule. Examples of such adapter-primers and the use thereof in accordance with the methods of the invention are shown in Example 25 herein. Such adapter-primers may be used to add one or more recombination sites or portions thereof to circular or linear nucleic acid molecules in a variety of contexts and by a variety of techniques, including but not limited to amplification (e.g., PCR), ligation (e.g., enzymatic or chemical/synthetic ligation), recombination (e.g., homologous or non-homologous (illegitimate) recombination) and the like.

**Library:** refers to a collection of nucleic acid molecules (circular or linear). In one embodiment, a library may comprise a plurality (i.e., two or more) of DNA molecules, which may or may not be from a common source organism, organ, tissue, or cell. In another embodiment, a library is representative of all or a portion or a significant portion of the DNA content of an organism (a "genomic" library), or a set of nucleic acid molecules representative of all or a portion or a significant portion of the expressed nucleic acid molecules (a cDNA library) in a

cell, tissue, organ or organism. A library may also comprise random sequences made by *de novo* synthesis, mutagenesis of one or more sequences and the like. Such libraries may or may not be contained in one or more vectors.

**Amplification:** refers to any *in vitro* method for increasing a number of copies of a nucleotide sequence with the use of a polymerase. Nucleic acid amplification results in the incorporation of nucleotides into a DNA and/or RNA molecule or primer thereby forming a new molecule complementary to a template. The formed nucleic acid molecule and its template can be used as templates to synthesize additional nucleic acid molecules. As used herein, one amplification reaction may consist of many rounds of replication. DNA amplification reactions include, for example, polymerase chain reaction (PCR). One PCR reaction may consist of 5-100 "cycles" of denaturation and synthesis of a DNA molecule.

**Oligonucleotide:** refers to a synthetic or natural molecule comprising a covalently linked sequence of nucleotides which are joined by a phosphodiester bond between the 3' position of the deoxyribose or ribose of one nucleotide and the 5' position of the deoxyribose or ribose of the adjacent nucleotide. This term may be used interchangeably herein with the terms "nucleic acid molecule" and "polynucleotide," without any of these terms necessarily indicating any particular length of the nucleic acid molecule to which the term specifically refers.

**Nucleotide:** refers to a base-sugar-phosphate combination. Nucleotides are monomeric units of a nucleic acid molecule (DNA and RNA). The term nucleotide includes ribonucleoside triphosphates ATP, UTP, CTG, GTP and deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives include, for example, [ $\alpha$ S]dATP, 7-deaza-dGTP and 7-deaza-dATP. The term nucleotide as used herein also refers to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrated examples of dideoxyribonucleoside triphosphates include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. According to the present invention, a "nucleotide" may be unlabeled or detectably labeled by well known techniques. Detectable labels include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels.



**Hybridization:** The terms "hybridization" and "hybridizing" refers to base pairing of two complementary single-stranded nucleic acid molecules (RNA and/or DNA) to give a double stranded molecule. As used herein, two nucleic acid molecules may be hybridized, although the base pairing is not completely complementary. Accordingly, mismatched bases do not prevent hybridization of two nucleic acid molecules provided that appropriate conditions, well known in the art, are used. In some aspects, hybridization is said to be under "stringent conditions." By "stringent conditions" as used herein is meant overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Other terms used in the fields of recombinant DNA technology and molecular and cell biology as used herein will be generally understood by one of ordinary skill in the applicable arts.

### *Overview*

Two reactions constitute the recombinational cloning system of the present invention, referred to herein as the "GATEWAY™ Cloning System," as depicted generally in Figure 1. The first of these reactions, the **LR Reaction** (Figure 2), which may also be referred to interchangeably herein as the **Destination Reaction**, is the main pathway of this system. The LR Reaction is a recombination reaction between an Entry vector or clone and a Destination Vector, mediated by a cocktail of recombination proteins such as the GATEWAY™ LR Clonase™ Enzyme Mix described herein. This reaction transfers nucleic acid molecules of interest (which may be genes, cDNAs, cDNA libraries, or fragments thereof) from the Entry Clone to an Expression Vector, to create an Expression Clone.

The sites labeled L, R, B, and P are respectively the attL, attR, attB, and attP recombination sites for the bacteriophage  $\lambda$  recombination proteins that constitute the Clonase cocktail (referred to herein variously as "Clonase" or



“GATEWAY™ LR Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attL x attR recombination reactions, as described herein) or “GATEWAY™ BP Clonase™ Enzyme Mix” (for recombination protein mixtures mediating attB x attP recombination reactions, as described herein)). The Recombinational Cloning reactions are equivalent to concerted, highly specific, cutting and ligation reactions. Viewed in this way, the recombination proteins cut to the left and right of the nucleic acid molecule of interest in the Entry Clone and ligate it into the Destination vector, creating a new Expression Clone.

The nucleic acid molecule of interest in an Expression Clone is flanked by the small attB1 and attB2 sites. The orientation and reading frame of the nucleic acid molecule of interest are maintained throughout the subcloning, because attL1 reacts only with attR1, and attL2 reacts only with attR2. Likewise, attB1 reacts only with attP1, and attB2 reacts only with attP2. Thus, the invention also relates to methods of controlled or directional cloning using the recombination sites of the invention (or portions thereof), including variants, fragments, mutants and derivatives thereof which may have altered or enhanced specificity. The invention also relates more generally to any number of recombination site partners or pairs (where each recombination site is specific for and interacts with its corresponding recombination site). Such recombination sites are preferably made by mutating or modifying the recombination site to provide any number of necessary specificities (e.g., attB1-10, attP1-10, attL1-10, attR1-10, etc.), non-limiting examples of which are described in detail in the Examples herein.

When an aliquot from the recombination reaction is transformed into host cells (e.g., *E. coli*) and spread on plates containing an appropriate selection agent, e.g., an antibiotic such as ampicillin with or without methicillin, cells that take up the desired clone form colonies. The unreacted Destination Vector does not give ampicillin-resistant colonies, even though it carries the ampicillin-resistance gene, because it contains a toxic gene, e.g., *ccdB*. Thus selection for ampicillin resistance selects for *E. coli* cells that carry the desired product, which usually comprise >90% of the colonies on the ampicillin plate.

To participate in the Recombinational (or “GATEWAY™”) Cloning Reaction, a nucleic acid molecule of interest first may be cloned into an Entry

Vector, creating an Entry Clone. Multiple options are available for creating Entry Clones, including: cloning of PCR sequences with terminal attB recombination sites into Entry Vectors; using the GATEWAY™ Cloning System recombination reaction; transfer of genes from libraries prepared in GATEWAY™ Cloning System vectors by recombination into Entry Vectors; and cloning of restriction enzyme-generated fragments and PCR fragments into Entry Vectors by standard recombinant DNA methods. These approaches are discussed in further detail herein.

A key advantage of the GATEWAY™ Cloning System is that a nucleic acid molecule of interest (or even a population of nucleic acid molecules of interest) present as an Entry Clone can be subcloned in parallel into one or more Destination Vectors in a simple reactions for anywhere from about 30 seconds to about 60 minutes (preferably about 1-60 minutes, about 1-45 minutes, about 1-30 minutes, about 2-60 minutes, about 2-45 minutes, about 2-30 minutes, about 1-2 minutes, about 30-60 minutes, about 45-60 minutes, or about 30-45 minutes). Longer reaction times (*e.g.*, 2-24 hours, or overnight) may increase recombination efficiency, particularly where larger nucleic acid molecules are used, as described in the Examples herein. Moreover, a high percentage of the colonies obtained carry the desired Expression Clone. This process is illustrated schematically in Figure 3, which shows an advantage of the invention in which the molecule of interest can be moved simultaneously or separately into multiple Destination Vectors. In the LR Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (*e.g.*, linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

The second major pathway of the GATEWAY™ Cloning System is the BP Reaction (Figure 4), which may also be referred to interchangeably herein as the Entry Reaction or the Gateward Reaction. The BP Reaction may recombine an Expression Clone with a Donor Plasmid (the counterpart of the byproduct in Figure 2). This reaction transfers the nucleic acid molecule of interest (which may have any of a variety of topologies, including linear, coiled, supercoiled, etc.) in the Expression Clone into an Entry Vector, to produce a new Entry Clone. Once this nucleic acid molecule of interest is cloned into an Entry

Vector, it can be transferred into new Expression Vectors, through the LR Reaction as described above. In the BP Reaction, one or both of the nucleic acid molecules to be recombined may have any topology (*e.g.*, linear, relaxed circular, nicked circular, supercoiled, etc.), although one or both are preferably linear.

5 A useful variation of the BP Reaction permits rapid cloning and expression of products of amplification (*e.g.*, PCR) or nucleic acid synthesis. Amplification (*e.g.*, PCR) products synthesized with primers containing terminal 25 bp attB sites serve as efficient substrates for the Gateway Cloning reaction. Such amplification products may be recombined with a Donor Vector to produce an Entry Clone (see  
10 Figure 7). The result is an Entry Clone containing the amplification fragment. Such Entry Clones can then be recombined with Destination Vectors -- through the LR Reaction -- to yield Expression Clones of the PCR product.

Additional details of the LR Reaction are shown in Figure 5A. The GATEWAY™ LR Clonase™ Enzyme Mix that mediates this reaction contains  
15 lambda recombination proteins Int (Integrase), Xis (Excisionase), and IHF (Integration Host Factor). In contrast, the GATEWAY™ BP Clonase™ Enzyme Mix, which mediates the BP Reaction (Figure 5B), comprises Int and IHF alone.

The recombination (att) sites of each vector comprise two distinct segments, donated by the parental vectors. The staggered lines dividing the two  
20 portions of each att site, depicted in Figures 5A and 5B, represent the seven-base staggered cut produced by Int during the recombination reactions. This structure is seen in greater detail in Figure 6, which displays the attB recombination sequences of an Expression Clone, generated by recombination between the attL1 and attL2 sites of an Entry Clone and the attR1 and attR2 sites of a Destination  
25 Vector.

The nucleic acid molecule of interest in the Expression Clone is flanked by attB sites: attB1 to the left (amino terminus) and attB2 to the right (carboxy terminus). The bases in attB1 to the left of the seven-base staggered cut produced by Int are derived from the Destination vector, and the bases to the right of the  
30 staggered cut are derived from the Entry Vector (see Figure 6). Note that the sequence is displayed in triplets corresponding to an open reading frame. If the reading frame of the nucleic acid molecule of interest cloned in the Entry Vector

is in phase with the reading frame shown for attB1, amino-terminal protein fusions can be made between the nucleic acid molecule of interest and any GATEWAY™ Cloning System Destination Vector encoding an amino-terminal fusion domain. Entry Vectors and Destination Vectors that enable cloning in all three reading frames are described in more detail herein, particularly in the Examples.

The LR Reaction allows the transfer of a desired nucleic acid molecule of interest into new Expression Vectors by recombining a Entry Clone with various Destination Vectors. To participate in the LR or Destination Reaction, however, a nucleic acid molecule of interest preferably is first converted to a Entry Clone. Entry Clones can be made in a number of ways, as shown in Figure 7.

One approach is to clone the nucleic acid molecule of interest into one or more of the Entry Vectors, using standard recombinant DNA methods, with restriction enzymes and ligase. The starting DNA fragment can be generated by restriction enzyme digestion or as a PCR product. The fragment is cloned between the attL1 and attL2 recombination sites in the Entry Vector. Note that a toxic or "death" gene (*e.g.*, *ccdB*), provided to minimize background colonies from incompletely digested Entry Vector, must be excised and replaced by the nucleic acid molecule of interest.

A second approach to making an Entry Clone (Figure 7) is to make a library (genomic or cDNA) in an Entry Vector, as described in detail herein. Such libraries may then be transferred into Destination Vectors for expression screening, for example in appropriate host cells such as yeast cells or mammalian cells.

A third approach to making Entry Clones (Figure 7) is to use Expression Clones obtained from cDNA molecules or libraries prepared in Expression Vectors. Such cDNAs or libraries, flanked by attB sites, can be introduced into a Entry Vector by recombination with a Donor Vector via the BP Reaction. If desired, an entire Expression Clone library can be transferred into the Entry Vector through the BP Reaction. Expression Clone cDNA libraries may also be constructed in a variety of prokaryotic and eukaryotic GATEWAY™-modified vectors (*e.g.*, the pEXP501 Expression Vector (see Figure 48), and 2-hybrid and

attB library vectors), as described in detail herein, particularly in the Examples below.

A fourth, and potentially most versatile, approach to making an Entry Clone (Figure 7) is to introduce a sequence for a nucleic acid molecule of interest into an Entry Vector by amplification (*e.g.*, PCR) fragment cloning. This method is diagramed in Figure 8. The DNA sequence first is amplified (for example, with PCR) as outlined in detail below and in the Examples herein, using primers containing one or more bp, two or more bp, three or more bp, four or more bp, five or more bp, preferably six or more bp, more preferably 6-25 bp (particularly 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25) bp of the attB nucleotide sequences (such as, but not limited to, those depicted in Figure 9), and optionally one or more, two or more, three or more, four or more, and most preferably four or five or more additional terminal nucleotide bases which preferably are guanines. The PCR product then may be converted to a Entry Clone by performing a BP Reaction, in which the attB-PCR product recombines with a Donor Vector containing one or more attP sites. Details of this approach and protocols for PCR fragment subcloning are provided in Examples 8 and 21-25.

A variety of Entry Clones may be produced by these methods, providing a wide array of cloning options; a number of specific Entry Vectors are also available commercially from Life Technologies, Inc. (Rockville, MD). The Examples herein provide a more in-depth description of selected Entry Vectors and details of their cloning sites. Choosing the optimal Entry Vector for a particular application is discussed in Example 4.

Entry Vectors and Destination Vectors should be constructed so that the amino-terminal region of a nucleic acid molecule of interest (*e.g.*, a gene, cDNA library or insert, or fragment thereof) will be positioned next to the attL1 site. Entry Vectors preferably contain the *rrnB* transcriptional terminator upstream of the attL1 site. This sequence ensures that expression of cloned nucleic acid molecules of interest is reliably "off" in *E. coli*, so that even toxic genes can be successfully cloned. Thus, Entry Clones may be designed to be transcriptionally silent. Note also that Entry Vectors, and hence Entry Clones, may contain the kanamycin antibiotic resistance (*kan<sup>r</sup>*) gene to facilitate selection of host cells



containing Entry Clones after transformation. In certain applications, however, Entry Clones may contain other selection markers, including but not limited to a gentamycin resistance ( $gen^r$ ) or tetracycline resistance ( $tet^r$ ) gene, to facilitate selection of host cells containing Entry Clones after transformation.

5        Once a nucleic acid molecule of interest has been cloned into an Entry Vector, it may be moved into a Destination Vector. The upper right portion of Figure 5A shows a schematic of a Destination Vector. The thick arrow represents some function (often transcription or translation) that will act on the nucleic acid molecule of interest in the clone. During the recombination reaction, the region  
10        between the attR1 and attR2 sites, including a toxic or "death" gene (*e.g.*, *ccdB*), is replaced by the DNA segment from the Entry Clone. Selection for recombinants that have acquired the ampicillin resistance ( $amp^r$ ) gene (carried on the Destination Vector) and that have also lost the death gene ensures that a high percentage (usually >90%) of the resulting colonies will contain the correct insert.

15        To move a nucleic acid molecule of interest into a Destination Vector, the Destination Vector is mixed with the Entry Clone comprising the desired nucleic acid molecule of interest, a cocktail of recombination proteins (*e.g.*, GATEWAY™ LR Clonase™ Enzyme Mix) is added, the mixture is incubated (preferably at about 25°C for about 60 minutes, or longer under certain  
20        circumstances, *e.g.* for transfer of large nucleic acid molecules, as described below) and any standard host cell (including bacterial cells such as *E. coli*; animal cells such as insect cells, mammalian cells, nematode cells and the like; plant cells; and yeast cells) strain is transformed with the reaction mixture. The host cell used will be determined by the desired selection (*e.g.*, *E. coli* DB3.1, available  
25        commercially from Life Technologies, Inc., allows survival of clones containing the *ccdB* death gene, and thus can be used to select for cointegrate molecules -- *i.e.*, molecules that are hybrids between the Entry Clone and Destination Vector). The Examples below provide further details and protocols for use of Entry and Destination Vectors in transferring nucleic acid molecules of interest and  
30        expressing RNAs or polypeptides encoded by these nucleic acid molecules in a variety of host cells.



The cloning system of the invention therefore offers multiple advantages:

- Once a nucleic acid molecule of interest is cloned into the GATEWAY™ Cloning System, it can be moved into and out of other vectors with complete fidelity of reading frame and orientation. That is, since the reactions proceed whereby attL1 on the Entry Clone recombines with attR1 on the Destination Vector, the directionality of the nucleic acid molecule of interest is maintained or may be controlled upon transfer from the Entry Clone into the Destination Vector. Hence, the GATEWAY™ Cloning System provides a powerful and easy method of directional cloning of nucleic acid molecule of interest.
- One-step cloning or subcloning: Mix the Entry Clone and the Destination Vector with Clonase, incubate, and transform.
- Clone PCR products readily by *in vitro* recombination, by adding attB sites to PCR primers. Then directly transfer these Entry Clones into Destination Vectors. This process may also be carried out in one step (see Examples below).
- Powerful selections give high reliability: >90% ( and often >99%) of the colonies contain the desired DNA in its new vector.
- One-step conversion of existing standard vectors into GATEWAY™ Cloning System vectors.
- Ideal for large vectors or those with few cloning sites.
- Recombination sites are short (25 bp), and may be engineered to contain no stop codons or secondary structures.
- Reactions may be automated, for high-throughput applications (*e.g.*, for diagnostic purposes or for therapeutic candidate screening).
- The reactions are economical: 0.3 µg of each DNA; no restriction enzymes, phosphatase, ligase, or gel purification. Reactions work well with miniprep DNA.
- Transfer multiple clones, and even libraries, into one or more Destination Vectors, in a single experiment.
- A variety of Destination Vectors may be produced, for applications including, but not limited to:

- Protein expression in *E. coli*: native proteins; fusion proteins with GST, His6, thioredoxin, etc., for purification, or one or more epitope tags; any promoter useful in expressing proteins in *E. coli* may be used, such as ptrc,  $\lambda P_L$ , and T7 promoters.
- Protein expression in eukaryotic cells: CMV promoter, baculovirus (with or without His6 tag), Semliki Forest virus, Tet regulation.
- DNA sequencing (all *lac* primers), RNA probes, phagemids (both strands)
- A variety of Entry Vectors (for recombinational cloning entry by standard recombinant DNA methods) may be produced:
  - Strong transcription stop just upstream, for genes toxic to *E. coli*.
  - Three reading frames.
  - With or without TEV protease cleavage site.
  - Motifs for prokaryotic and / or eukaryotic translation.
  - Compatible with commercial cDNA libraries.
- Expression Clone cDNA (*attB*) libraries, for expression screening, including 2-hybrid libraries and phage display libraries, may also be constructed.

### ***Recombination Site Sequences***

In one aspect, the invention relates to nucleic acid molecules, which may or may not be isolated nucleic acid molecules, comprising one or more nucleotide sequences encoding one or more recombination sites or portions thereof. In particular, this aspect of the invention relates to such nucleic acid molecules comprising one or more nucleotide sequences encoding *attB*, *attP*, *attL*, or *attR*, or portions of these recombination site sequences. The invention also relates to mutants, derivatives, and fragments of such nucleic acid molecules. Unless otherwise indicated, all nucleotide sequences that may have been determined by sequencing a DNA molecule herein were determined using manual or automated DNA sequencing, such as dideoxy sequencing, according to methods that are routine to one of ordinary skill in the art (Sanger, F., and Coulson, A.R., *J. Mol. Biol.* 94:444-448 (1975); Sanger, F., et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)). All amino acid sequences of polypeptides encoded by DNA

molecules determined herein were predicted by conceptual translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by these approaches, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by such methods are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Unless otherwise indicated, each "nucleotide sequence" set forth herein is presented as a sequence of deoxyribonucleotides (abbreviated A, G, C and T). However, by "nucleotide sequence" of a nucleic acid molecule or polynucleotide is intended, for a DNA molecule or polynucleotide, a sequence of deoxyribonucleotides, and for an RNA molecule or polynucleotide, the corresponding sequence of ribonucleotides (A, G, C and U), where each thymidine deoxyribonucleotide (T) in the specified deoxyribonucleotide sequence is replaced by the ribonucleotide uridine (U). Thus, the invention relates to sequences of the invention in the form of DNA or RNA molecules, or hybrid DNA/RNA molecules, and their corresponding complementary DNA, RNA, or DNA/RNA strands.

In a first such aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAAGCAGGCT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB1*, or mutants, fragments, variants or derivatives thereof. As one of ordinary skill will appreciate, however, certain mutations, insertions, or deletions of one or more bases in the *attB1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional

integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB1* sequence are encompassed within the scope of the invention.

In a related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attB2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attB2* nucleotide sequence having the sequence set forth in Figure 9, such as: ACCCAGCTTTCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attB2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attB2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attB2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule containing *attB1* and *attB2* sites (the vector pEXP501, also known as pCMVSPORT6; see Figure 48), *E. coli* DB3.1(pCMVSPORT6), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30108. The *attB1* and *attB2* sites within the deposited nucleic acid molecule are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP1* nucleotide sequence having the sequence set forth in Figure 9, such as: TACAGGTCACATAATACCATCTAAGTAGTTGATTCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACAGGTCACATATCAGTCAAAATAA-

AATCATTATTTG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attP2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attP2* nucleotide sequence having the sequence set forth in Figure 9, such as: CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTG-CAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTT-GTACAAGAAAGCTGAACGAGAAACGTAAAATGATA-TAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG-ACTACATAATACTGTAAACACAACATATCCAGTCACTATGAATCAA-CTACTTAGATGGTATTAGTGACCTGTA, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attP2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attP2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attP2* sequence are encompassed within the scope of the invention.

A recombinant host cell comprising a nucleic acid molecule (the *attP* vector pDONR201, also known as pENTR21-*attPkan* or pAttPkan; see Figure 49) containing *attP1* and *attP2* sites, *E. coli* DB3.1(pAttPkan) (also called *E. coli* DB3.1(pAHKan)), was deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit No. NRRL B-30099. The *attP1* and *attP2* sites within the deposited nucleic acid molecule are contained in nucleic acid

cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR1*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR1* nucleotide sequence having the sequence set forth in Figure 9, such as: ACAAGTTTGTACAAAAAGCTGAACGAG-AAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-AAAAACAGACTACATAATACTGTAAAACACAACATATCCAGTCA-CTATG, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR1*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR1* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR1* sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding *attR2*, or mutants, fragments, variants or derivatives thereof. Such nucleic acid molecules may comprise an *attR2* nucleotide sequence having the sequence set forth in Figure 9, such as: GCAGGTCGACCATAGTGACTGGATAT-GTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA-ATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTT-TCTTGTACAAAGTGGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attR2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attR2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attR2* sequence are encompassed within the scope of the invention.



Recombinant host cell strains containing attR1 sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pEZC15101) (reading frame A; see Figure 64A), *E. coli* DB3.1(pEZC15102) (reading frame B; see Figure 64B), and *E. coli* DB3.1(pEZC15103) (reading frame C; see Figure 64C), and containing corresponding attR2 sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30103, NRRL B-30104, and NRRL B-30105, respectively. The attR1 and attR2 sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL1, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL1 nucleotide sequence having the sequence set forth in Figure 9, such as: CAA ATA ATG ATT TTA TTT TGA CTG ATA GTG ACC TGT TCG TTG CAA CAA ATT GAT AAG CAA TGC TTT TTT ATA ATG CCA ACT TTG TAC AAA AAA GCA GGC T, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for attL1, or mutants, fragments, variants or derivatives thereof. As noted above for attB1, certain mutations, insertions, or deletions of one or more bases in the attL1 sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the attL1 sequence are encompassed within the scope of the invention.

In another related aspect, the invention provides nucleic acid molecules comprising one or more nucleotide sequences encoding attL2, or mutants, fragments, variants and derivatives thereof. Such nucleic acid molecules may comprise an attL2 nucleotide sequence having the sequence set forth in Figure 9, such as: C AAA TAA TGA TTT TAT TTT GAC TGA TAG TGA CCT GTT CGT TGC AAC AAA TTG ATA AGC AAT GCT TTC TTA TAA TGC CAA

CTT TGT ACA AGA AAG CTG GGT, or a nucleotide sequence complementary to the nucleotide sequence set forth in Figure 9 for *attL2*, or mutants, fragments, variants or derivatives thereof. As noted above for *attB1*, certain mutations, insertions, or deletions of one or more bases in the *attL2* sequence contained in the nucleic acid molecules of the invention may be made without compromising the structural and functional integrity of these molecules; hence, nucleic acid molecules comprising such mutations, insertions, or deletions in the *attL2* sequence are encompassed within the scope of the invention.

Recombinant host cell strains containing *attL1* sites apposed to cloning sites in reading frame A, reading frame B, and reading frame C, *E. coli* DB3.1(pENTR1A) (reading frame A; see Figure 10), *E. coli* DB3.1(pENTR2B) (reading frame B; see Figure 11), and *E. coli* DB3.1(pENTR3C) (reading frame C; see Figure 12), and containing corresponding *attL2* sites, were deposited on February 27, 1999, with the Collection, Agricultural Research Culture Collection (NRRL), 1815 North University Street, Peoria, Illinois 61604 USA, as Deposit Nos. NRRL B-30100, NRRL B-30101, and NRRL B-30102, respectively. The *attL1* and *attL2* sites within the deposited nucleic acid molecules are contained in nucleic acid cassettes in association with one or more additional functional sequences as described in more detail below.

Each of the recombination site sequences described herein or portions thereof, or the nucleotide sequence cassettes contained in the deposited clones, may be cloned or inserted into a vector of interest (for example, using the recombinational cloning methods described herein and/or standard restriction cloning techniques that are routine in the art) to generate, for example, Entry Vectors or Destination Vectors which may be used to transfer a desired segment of a nucleic acid molecule of interest (e.g., a gene, cDNA molecule, or cDNA library) into a desired vector or into a host cell.

Using the information provided herein, such as the nucleotide sequences for the recombination site sequences described herein, an isolated nucleic acid molecule of the present invention encoding one or more recombination sites or portions thereof may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Preferred such

methods include PCR-based cloning methods, such as reverse transcriptase-PCR (RT-PCR) using primers such as those described herein and in the Examples below. Alternatively, vectors comprising the cassettes containing the recombination site sequences described herein are available commercially from Life Technologies, Inc. (Rockville, MD).

The invention is also directed to nucleic acid molecules comprising one or more of the recombination site sequences or portions thereof and one or more additional nucleotide sequences, which may encode functional or structural sites such as one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (which may be promoters, enhancers, repressors, and the like), one or more translational signals (*e.g.*, secretion signal sequences), one or more origins of replication, one or more fusion partner peptides (particularly glutathione S-transferase (GST), hexahistidine (His<sub>6</sub>), and thioredoxin (Trx)), one or more selection markers or modules, one or more nucleotide sequences encoding localization signals such as nuclear localization signals or secretion signals, one or more origins of replication, one or more protease cleavage sites, one or more genes or portions of genes encoding a protein or polypeptide of interest, and one or more 5' polynucleotide extensions (particularly an extension of guanine residues ranging in length from about 1 to about 20, from about 2 to about 15, from about 3 to about 10, from about 4 to about 10, and most preferably an extension of 4 or 5 guanine residues at the 5' end of the recombination site nucleotide sequence. The one or more additional functional or structural sequences may or may not flank one or more of the recombination site sequences contained on the nucleic acid molecules of the invention.

In some nucleic acid molecules of the invention, the one or more nucleotide sequences encoding one or more additional functional or structural sites may be operably linked to the nucleotide sequence encoding the recombination site. For example, certain nucleic acid molecules of the invention may have a promoter sequence operably linked to a nucleotide sequence encoding a recombination site or portion thereof of the invention, such as a T7 promoter, a phage lambda PL

promoter, an *E. coli lac*, *trp* or *tac* promoter, and other suitable promoters which will be familiar to the skilled artisan.

Nucleic acid molecules of the present invention, which may be isolated nucleic acid molecules, may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically, or in the form of DNA-RNA hybrids. The nucleic acid molecules of the invention may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. The nucleic acid molecules of the invention may also have a number of topologies, including linear, circular, coiled, or supercoiled.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells, and those DNA molecules purified (partially or substantially) from a solution whether produced by recombinant DNA or synthetic chemistry techniques. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention.

The present invention further relates to mutants, fragments, variants and derivatives of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of one or more recombination sites. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (see Lewin, B., ed., *Genes II*, , John Wiley & Sons, New York (1985)). Non-naturally occurring variants may be produced using art-known mutagenesis techniques, such as those described hereinbelow.

Such variants include those produced by nucleotide substitutions, deletions or additions or portions thereof, or combinations thereof. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding

regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the encoded polypeptide(s) or portions thereof, and which also do not substantially alter the reactivities of the recombination site nucleic acid sequences in recombination reactions. Also especially preferred in this regard are conservative substitutions.

Particularly preferred mutants, fragments, variants, and derivatives of the nucleic acid molecules of the invention include, but are not limited to, insertions, deletions or substitutions of one or more nucleotide bases within the 15 bp core region (GCTTTTTTATACTAA) which is identical in all four wildtype lambda *att* sites, *attB*, *attP*, *attL* and *attR* (see U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, which describes the core region in further detail, and the disclosures of which are incorporated herein by reference in their entireties). Analogously, the core regions in *attB1*, *attP1*, *attL1* and *attR1* are identical to one another, as are the core regions in *attB2*, *attP2*, *attL2* and *attR2*. Particularly preferred in this regard are nucleic acid molecules comprising insertions, deletions or substitutions of one or more nucleotides within the seven bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) that occurs within this 15 bp core region (GCTTTTTTATACTAA). Examples of such preferred mutants, fragments, variants and derivatives according to this aspect of the invention include, but are not limited to, nucleic acid molecules in which the thymine at position 1 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 2 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the thymine at position 3 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or adenine; in which the adenine at position 4 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; in which the thymine at position 5 of the seven bp overlap region has been deleted or substituted with a



5 guanine, cytosine, or adenine; in which the adenine at position 6 of the seven bp overlap region has been deleted or substituted with a guanine, cytosine, or thymine; and in which the cytosine at position 7 of the seven bp overlap region has been deleted or substituted with a guanine, thymine, or adenine; or any combination of one or more such deletions and/or substitutions within this seven bp overlap region. As described in detail in Example 21 herein, mutants of the nucleic acid molecules of the invention in which substitutions have been made within the first three positions of the seven bp overlap (TTTAATAC) have been found in the present invention to strongly affect the specificity of recombination, mutant nucleic acid molecules in which substitutions have been made in the last four positions (TTTAATAC) only partially alter recombination specificity, and mutant nucleic acid molecules comprising nucleotide substitutions outside of the seven bp overlap, but elsewhere within the 15 bp core region, do not affect specificity of recombination but do influence the efficiency of recombination.

15 Hence, in an additional aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that affect recombination specificity, particularly one or more nucleotide sequences that may correspond substantially to the seven base pair overlap within the 15 bp core region, having one or more mutations that affect recombination specificity. Particularly preferred such molecules may comprise a consensus sequence (described in detail in Example 21 herein) such as NNNATAC, wherein "N" refers to any nucleotide (*i.e.*, may be A, G, T/U or C), with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

25 In a related aspect, the present invention is also directed to nucleic acid molecules comprising one or more recombination site nucleotide sequences that enhance recombination efficiency, particularly one or more nucleotide sequences that may correspond substantially to the core region and having one or more mutations that enhance recombination efficiency. By sequences or mutations that "enhance recombination efficiency" is meant a sequence or mutation in a recombination site, preferably in the core region (*e.g.*, the 15 bp core region of *att* recombination sites), that results in an increase in cloning efficiency (typically

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measured by determining successful cloning of a test sequence, e.g., by determining CFU/ml for a given cloning mixture) when recombining molecules comprising the mutated sequence or core region as compared to molecules that do not comprise the mutated sequence or core region (e.g., those comprising a wildtype recombination site core region sequence). More specifically, whether or not a given sequence or mutation enhances recombination efficiency may be determined using the sequence or mutation in recombinational cloning as described herein, and determining whether the sequence or mutation provides enhanced recombinational cloning efficiency when compared to a non-mutated (e.g., wildtype) sequence. Methods of determining preferred cloning efficiency-enhancing mutations for a number of recombination sites, particularly for *att* recombination sites, are described herein, for example in Examples 22-25. Examples of preferred such mutant recombination sites include but are not limited to the *attL* consensus core sequence of caacttntntnnannaagttg (wherein "n" represents any nucleotide), for example the *attL5* sequence agcctgctttattataactaagttggcatta and the *attL6* sequence agcctgcttttttatattaagttggcatta; the *attB1.6* sequence ggggacaactttgtacaaaaagttggct; the *attB2.2* sequence ggggacaactttgtacaagaagctgggt; and the *attB2.10* sequence ggggacaactttgtacaagaagttgggt. Those of skill in the art will appreciate that, in addition to the core region, other portions of the *att* site may affect the efficiency of recombination. There are five so-called arm binding sites for the integrase protein in the bacteriophage lambda *attP* site, two in *attR* (P1 and P2), and three in *attL* (P'1, P'2 and P'3). Compared to the core binding sites, the integrase protein binds to arm sites with high affinity and interacts with core and arm sites through two different domains of the protein. As with the core binding site a consensus sequence for the arm binding site consisting of C/AAGTCACTAT has been inferred from sequence comparison of the five arm binding sites and seven non-*att* sites (Ross and Landy, *Proc. Natl. Acad. Sci. USA* 79:7724-7728 (1982)). Each arm site has been mutated and tested for its effect in the excision and integration reactions (Numrych *et al.*, *Nucl. Acids Res.* 18:3953 (1990)). Hence, specific sites are utilized in each reaction in different ways, namely, the P1 and P'3

5 sites are essential for the integration reaction whereas the other three sites are dispensable to the integration reaction to varying degrees. Similarly, the P2, P'1 and P'2 sites are most important for the excision reaction, whereas P1 and P'3 are completely dispensable. Interestingly, when P2 is mutated the integration reaction occurs more efficiently than with the wild type attP site. Similarly, when P1 and P'3 are mutated the excision reaction occurs more efficiently. The stimulatory effect of mutating integrase arm binding sites can be explained by removing sites that compete or inhibit a specific recombination pathway or that function in a reaction that converts products back to starting substrates. In fact there is evidence for an XIS-independent LR reaction (Abremski and Gottesman, *J. Mol. Biol.* 153:67-78 (1981)). Thus, in addition to modifications in the core region of the att site, the present invention contemplates the use of att sites containing one or more modifications in the integrase arm-type binding sites. In some preferred embodiments, one or more mutations may be introduced into one or more of the P1, P'1, P2, P'2 and P'3 sites. In some preferred embodiments, multiple mutations may be introduced into one or more of these sites. Preferred such mutations include those which increase the recombination *in vitro*. For example, in some embodiments mutations may be introduced into the arm-type binding sites such that integrative recombination, corresponding to the BP reaction, is enhanced. In other embodiments, mutations may be introduced into the arm-type binding sites such that excisive recombination, corresponding to the LR reaction, is enhanced. Of course, based on the guidance contained herein, particularly in the construction and evaluation of effects of mutated recombination sites upon recombinational specificity and efficiency, analogous mutated or engineered sequences may be produced for other recombination sites described herein (including but not limited to *lox*, FRT, and the like) and used in accordance with the invention. For example, much like the mutagenesis strategy used to select core binding sites that enhance recombination efficiency, similar strategies can be employed to select changes in the arms of attP, attL and attR, and in analogous sequences in other recombination sites such as *lox*, FRT and the like, that enhance recombination efficiency. Hence, the construction and evaluation of such mutants is well within the abilities of those of ordinary skill in the art without undue experimentation.

One suitable methodology for preparing and evaluating such mutations is found in Numrych, *et al.*, (1990) *Nucleic Acids Research* 18(13): 3953-3959.

Other mutant sequences and nucleic acid molecules that may be suitable to enhance recombination efficiency will be apparent from the description herein, or may be easily determined by one of ordinary skill using only routine experimentation in molecular biology in view of the description herein and information that is readily available in the art

Since the genetic code is well known in the art, it is also routine for one of ordinary skill in the art to produce degenerate variants of the nucleic acid molecules described herein without undue experimentation. Hence, nucleic acid molecules comprising degenerate variants of nucleic acid sequences encoding the recombination sites described herein are also encompassed within the scope of the invention.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 50% identical, at least 60% identical, at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to the nucleotide sequences of the seven bp overlap region within the 15 bp core region of the recombination sites described herein, or the nucleotide sequences of *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* as set forth in Figure 9 (or portions thereof), or a nucleotide sequence complementary to any of these nucleotide sequences, or fragments, variants, mutants, and derivatives thereof.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a particular recombination site or portion thereof is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations (*e.g.*, insertions, substitutions, or deletions) per each 100 nucleotides of the reference nucleotide sequence encoding the recombination site. For example, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference *attB1* nucleotide sequence, up to 5% of the nucleotides in the *attB1* reference sequence may be

deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the *attB1* reference sequence may be inserted into the *attB1* reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, a given recombination site nucleotide sequence or portion thereof can be determined conventionally using known computer programs such as DNAsis software (Hitachi Software, San Bruno, California) for initial sequence alignment followed by ESEE version 3.0 DNA/protein sequence software (cabot@trog.mbb.sfu.ca) for multiple sequence alignments. Alternatively, such determinations may be accomplished using the BESTFIT program (Wisconsin Sequence Analysis Package, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711), which employs a local homology algorithm (Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981)) to find the best segment of homology between two sequences. When using DNAsis, ESEE, BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present invention is directed to nucleic acid molecules at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences as set forth in Figure 9, or to the nucleotide sequence of the deposited clones, irrespective of whether they encode particular functional polypeptides. This is because even where a particular nucleic acid molecule does not encode a particular functional polypeptide, one of skill in the art would still know how to use the nucleic acid

molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer.

5 Mutations can also be introduced into the recombination site nucleotide sequences for enhancing site specific recombination or altering the specificities of the reactants, etc. Such mutations include, but are not limited to: recombination sites without translation stop codons that allow fusion proteins to be encoded; recombination sites recognized by the same proteins but differing in base sequence such that they react largely or exclusively with their homologous partners allowing multiple reactions to be contemplated; and mutations that prevent hairpin formation of recombination sites. Which particular reactions take place can be specified by which particular partners are present in the reaction mixture.

10 There are well known procedures for introducing specific mutations into nucleic acid sequences. A number of these are described in Ausubel, F.M. *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1989-1996). Mutations can be designed into oligonucleotides, which can be used to modify existing cloned sequences, or in amplification reactions. Random mutagenesis can also be employed if appropriate selection methods are available to isolate the desired mutant DNA or RNA. The presence of the desired mutations can be confirmed by sequencing the nucleic acid by well known methods.

20 The following non-limiting methods can be used to modify or mutate a given nucleic acid molecule encoding a particular recombination site to provide mutated sites that can be used in the present invention:

- 25 1. By recombination of two parental DNA sequences by site-specific (e.g. attL and attR to give attP) or other (e.g. homologous) recombination mechanisms where the parental DNA segments contain one or more base alterations resulting in the final mutated nucleic acid molecule;
2. By mutation or mutagenesis (site-specific, PCR, random, spontaneous, etc) directly of the desired nucleic acid molecule;
- 30 3. By mutagenesis (site-specific, PCR, random, spontaneous, etc) of parental DNA sequences, which are recombined to generate a desired nucleic acid molecule;



4. By reverse transcription of an RNA encoding the desired core sequence;  
and

5. By *de novo* synthesis (chemical synthesis) of a sequence having the desired  
base changes, or random base changes followed by sequencing or  
functional analysis according to methods that are routine in the art.

The functionality of the mutant recombination sites can be demonstrated in  
ways that depend on the particular characteristic that is desired. For example, the  
lack of translation stop codons in a recombination site can be demonstrated by  
expressing the appropriate fusion proteins. Specificity of recombination between  
homologous partners can be demonstrated by introducing the appropriate  
molecules into *in vitro* reactions, and assaying for recombination products as  
described herein or known in the art. Other desired mutations in recombination  
sites might include the presence or absence of restriction sites, translation or  
transcription start signals, protein binding sites, particular coding sequences, and  
other known functionalities of nucleic acid base sequences. Genetic selection  
schemes for particular functional attributes in the recombination sites can be used  
according to known method steps. For example, the modification of sites to  
provide (from a pair of sites that do not interact) partners that do interact could  
be achieved by requiring deletion, via recombination between the sites, of a DNA  
sequence encoding a toxic substance. Similarly, selection for sites that remove  
translation stop sequences, the presence or absence of protein binding sites, etc.,  
can be easily devised by those skilled in the art.

Accordingly, the present invention also provides a nucleic acid molecule,  
comprising at least one DNA segment having at least one, and preferably at least  
two, engineered recombination site nucleotide sequences of the invention flanking  
a selectable marker and/or a desired DNA segment, wherein at least one of said  
recombination site nucleotide sequences has at least one engineered mutation that  
enhances recombination *in vitro* in the formation of a Cointegrate DNA or a  
Product DNA. Such engineered mutations may be in the core sequence of the  
recombination site nucleotide sequence of the invention; see U.S. Application Nos.  
08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent  
No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed



October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties.

While in the preferred embodiment the recombination sites differ in sequence and do not interact with each other, it is recognized that sites comprising the same sequence, which may interact with each other, can be manipulated or engineered to inhibit recombination with each other. Such conceptions are considered and incorporated herein. For example, a protein binding site (*e.g.*, an antibody-binding site, a histone-binding site, an enzyme-binding site, or a binding site for any nucleic acid molecule-binding protein) can be engineered adjacent to one of the sites. In the presence of the protein that recognizes the engineered site, the recombinase fails to access the site and another recombination site in the nucleic acid molecule is therefore used preferentially. In the cointegrate this site can no longer react since it has been changed, *e.g.*, from attB to attL. During or upon resolution of the cointegrate, the protein can be inactivated (*e.g.*, by antibody, heat or a change of buffer) and the second site can undergo recombination.

The nucleic acid molecules of the invention can have at least one mutation that confers at least one enhancement of said recombination, said enhancement selected from the group consisting of substantially (i) favoring integration; (ii) favoring recombination; (iii) relieving the requirement for host factors; (iv) increasing the efficiency of said Cointegrate DNA or Product DNA formation; (v) increasing the specificity of said Cointegrate DNA or Product DNA formation; and (vi) adding or deleting protein binding sites.

In other embodiments, the nucleic acid molecules of the invention may be PCR primer molecules, which comprise one or more of the recombination site sequences described herein or portions thereof, particularly those shown in Figure 9 (or sequences complementary to those shown in Figure 9), or mutants, fragments, variants or derivatives thereof, attached at the 3' end to a target-specific template sequence which specifically interacts with a target nucleic acid molecule which is to be amplified. Primer molecules according to this aspect of the invention may further comprise one or more, (*e.g.*, 1, 2, 3, 4, 5, 10, 20, 25, 50, 100, 500, 1000, or more) additional bases at their 5' ends, and preferably comprise one or more (particularly four or five) additional bases, which are preferably

guanines, at their 5' ends, to increase the efficiency of the amplification products incorporating the primer molecules in the recombinational cloning system of the invention. Such nucleic acid molecules and primers are described in detail in the examples herein, particularly in Examples 22-25.

5 Certain primers of the invention may comprise one or more nucleotide deletions in the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* sequences as set forth in Figure 9. In one such aspect, for example, *attB2* primers may be constructed in which one or more of the first four nucleotides at the 5' end of the *attB2* sequence shown in Figure 9 have been deleted. Primers according to this aspect of the invention may therefore have the sequence:

10 (*attB2*(-1)): CCCAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n

(*attB2*(-2)): CCAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n

(*attB2*(-3)): CAGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n

(*attB2*(-4)): AGCTTTCTTGTACAAAGTGGTnnnnnnnnnnnnnn . . . n,

15 wherein "nnnnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (e.g., a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

20 The primer nucleic acid molecules according to this aspect of the invention may be produced synthetically by attaching the recombination site sequences depicted in Figure 9, or portions thereof, to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art. Alternatively, additional primer nucleic acid molecules of the invention may be produced synthetically by adding one or more nucleotide bases, which preferably correspond to one or more, preferably five or more, and more preferably six or more, contiguous nucleotides of the *att* nucleotide sequences described herein (see, e.g., Example 20 herein; see also U.S. Application Nos. 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of which are all incorporated herein by reference in their entireties), to the 5' end of a standard PCR target-specific primer according to methods that are well-known in the art, to provide

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primers having the specific nucleotide sequences described herein. As noted above, primer nucleic acid molecules according to this aspect of the invention may also optionally comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four or five guanines at their 5' ends. In one particularly preferred such aspect, the primer nucleic acid molecules of the invention may comprise one or more, preferably five or more, more preferably six or more, still more preferably 6-18 or 6-25, and most preferably 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25, contiguous nucleotides or bp of the *attB1* or *attB2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (e.g., a gene-specific) primer molecule. Primer nucleic acid molecules according to this aspect of the invention include, but are not limited to, *attB1*- and *attB2*-derived primer nucleic acid molecules having the following nucleotide sequences:

ACAAGTTTGTACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n

ACCACTTTGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n

TGTACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n

TGTACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n

ACAAAAAAGCAGGCT-nnnnnnnnnnnnn . . . n

ACAAGAAAGCTGGGT-nnnnnnnnnnnnn . . . n

AAAAAGCAGGCT-nnnnnnnnnnnnn . . . n

AGAAAGCTGGGT-nnnnnnnnnnnnn . . . n

AAAAGCAGGCT-nnnnnnnnnnnnn . . . n

GAAAGCTGGGT-nnnnnnnnnnnnn . . . n

AAAGCAGGCT-nnnnnnnnnnnnn . . . n

AAAGCTGGGT-nnnnnnnnnnnnn . . . n

AAGCAGGCT-nnnnnnnnnnnnn . . . n

AAGCTGGGT-nnnnnnnnnnnnn . . . n

AGCAGGCT-nnnnnnnnnnnnn . . . n

AGCTGGGT-nnnnnnnnnnnnn . . . n

GCAGGCT-nnnnnnnnnnnnn . . . n

GCTGGGT-nnnnnnnnnnnnn . . . n

CAGGCT-nnnnnnnnnnnnn . . . n

CTGGGT-nnnnnnnnnnnnn . . . n,

wherein "nnnnnnnnnnnn . . . n" at the 3' end of the primer represents a target-specific sequence of any length, for example from one base up to all of the bases of a target nucleic acid molecule (e.g., a gene) or a portion thereof, the sequence and length which will depend upon the identity of the target nucleic acid molecule which is to be amplified.

Of course, it will be apparent to one of ordinary skill from the teachings contained herein that additional primer nucleic acid molecules analogous to those specifically described herein may be produced using one or more, preferably five or more, more preferably six or more, still more preferably ten or more, 15 or more, 20 or more, 25 or more, 30 or more, etc. (through to and including all) of the contiguous nucleotides or bp of the *attP1*, *attP2*, *attL1*, *attL2*, *attR1* or *attR2* nucleotide sequences depicted in Figure 9 (or nucleotides complementary thereto), linked to the 5' end of a target-specific (e.g., a gene-specific) primer molecule. As noted above, such primer nucleic acid molecules may optionally further comprise one, two, three, four, five, or more additional nucleotide bases at their 5' ends, and preferably will comprise four guanines at their 5' ends. Other primer molecules comprising the *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* sequences depicted in Figure 9, or portions thereof, may be made by one of ordinary skill without resorting to undue experimentation in accordance with the guidance provided herein.

The primers of the invention described herein are useful in producing PCR fragments having a nucleic acid molecule of interest flanked at each end by a recombination site sequence (as described in detail below in Example 9), for use in cloning of PCR-amplified DNA fragments using the recombination system of the invention (as described in detail below in Examples 8, 19 and 21-25).

### Vectors

The invention also relates to vectors comprising one or more of the nucleic acid molecules of the invention, as described herein. In accordance with the invention, any vector may be used to construct the vectors of the invention. In

particular, vectors known in the art and those commercially available (and variants or derivatives thereof) may in accordance with the invention be engineered to include one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), or mutants, fragments, or derivatives thereof, for use in the methods of the invention. Such vectors may be obtained from, for example, Vector Laboratories Inc., InVitrogen, Promega, Novagen, New England Biolabs, Clontech, Roche, Pharmacia, EpiCenter, OriGenes Technologies Inc., Stratagene, Perkin Elmer, Pharmingen, Life Technologies, Inc., and Research Genetics. Such vectors may then for example be used for cloning or subcloning nucleic acid molecules of interest. General classes of vectors of particular interest include prokaryotic and/or eukaryotic cloning vectors, Expression Vectors, fusion vectors, two-hybrid or reverse two-hybrid vectors, shuttle vectors for use in different hosts, mutagenesis vectors, transcription vectors, vectors for receiving large inserts and the like.

Other vectors of interest include viral origin vectors (M13 vectors, bacterial phage  $\lambda$  vectors, bacteriophage P1 vectors, adenovirus vectors, herpesvirus vectors, retrovirus vectors, phage display vectors, combinatorial library vectors), high, low, and adjustable copy number vectors, vectors which have compatible replicons for use in combination in a single host (pACYC184 and pBR322) and eukaryotic episomal replication vectors (pCDM8).

Particular vectors of interest include prokaryotic Expression Vectors such as pcDNA II, pSL301, pSE280, pSE380, pSE420, pTrcHisA, B, and C, pRSET A, B, and C (Invitrogen, Inc.), pGEMEX-1, and pGEMEX-2 (Promega, Inc.), the pET vectors (Novagen, Inc.), pTrc99A, pKK223-3, the pGEX vectors, pEZZ18, pRIT2T, and pMC1871 (Pharmacia, Inc.), pKK233-2 and pKK388-1 (Clontech, Inc.), and pProEx-HT (Life Technologies, Inc.) and variants and derivatives thereof. Destination Vectors can also be made from eukaryotic Expression Vectors such as pFastBac, pFastBac HT, pFastBac DUAL, pSFV, and pTet-Splice (Life Technologies, Inc.), pEUK-C1, pPUR, pMAM, pMAMneo, pBI101, pBI121, pDR2, pCMVEBNA, and pYACneo (Clontech), pSVK3, pSVL, pMSG, pCH110, and pKK232-8 (Pharmacia, Inc.), p3'SS, pXT1, pSG5, pPbac, pMbac, pMC1neo, and pOG44 (Stratagene, Inc.), and pYES2, pAC360, pBlueBacHis A,

B, and C, pVL1392, pBsueBacIII, pCDM8, pcDNA1, pZeoSV, pcDNA3 pREP4, pCEP4, and pEBVHis (Invitrogen, Inc.) and variants or derivatives thereof.

Other vectors of particular interest include pUC18, pUC19, pBlueScript, pSPORT, cosmid, phagemids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes), MACs (mammalian artificial chromosomes), pQE70, pQE60, pQE9 (Quiagen), pBS vectors, PhageScript vectors, BlueScript vectors, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene), pcDNA3 (Invitrogen), pGEX, pTrsfus, pTrc99A, pET-5, pET-9, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia), pSPORT1, pSPORT2, pCMVSPORT2.0 and pSV-SPORT1 (Life Technologies, Inc.) and variants or derivatives thereof.

Additional vectors of interest include pTrxFus, pThioHis, pLEX, pTrcHis, pTrcHis2, pRSET, pBlueBacHis2, pcDNA3.1/His, pcDNA3.1(-)/Myc-His, pSecTag, pEBVHis, pPIC9K, pPIC3.5K, pAO815, pPICZ, pPICZ $\alpha$ , pGAPZ, pGAPZ $\alpha$ , pBlueBac4.5, pBlueBacHis2, pMelBac, pSinRep5, pSinHis, pIND, pIND(SP1), pVgRXX, pcDNA2.1, pYES2, pZER01.1, pZER0-2.1, pCR-Blunt, pSE280, pSE380, pSE420, pVL1392, pVL1393, pCDM8, pcDNA1.1, pcDNA1.1/Amp, pcDNA3.1, pcDNA3.1/Zeo, pSe,SV2, pRc/CMV2, pRc/RSV, pREP4, pREP7, pREP8, pREP9, pREP10, pCEP4, pEBVHis, pCR3.1, pCR2.1, pCR3.1-Uni, and pCRBac from Invitrogen;  $\lambda$ ExCell,  $\lambda$ gt11, pTrc99A, pKK223-3, pGEX-1 $\lambda$ T, pGEX-2T, pGEX-2TK, pGEX-4T-1, pGEX-4T-2, pGEX-4T-3, pGEX-3X, pGEX-5X-1, pGEX-5X-2, pGEX-5X-3, pEZZ18, pRIT2T, pMC1871, pSVK3, pSVL, pMSG, pCH110, pKK232-8, pSL1180, pNEO, and pUC4K from Pharmacia; pSCREEN-1b(+), pT7Blue(R), pT7Blue-2, pCITE-4abc(+), pOCUS-2, pTag, pET-32LIC, pET-30LIC, pBAC-2cpLIC, pBACgus-2cp LIC, pT7Blue-2 LIC, pT7Blue-2,  $\lambda$ SCREEN-1,  $\lambda$ BlueSTAR, pET-3abcd, pET-7abc, pET9abcd, pET11abcd, pET12abc, pET-14b, pET-15b, pET-16b, pET-17b-pET-17xb, pET-19b, pET-20b(+), pET-21abcd(+), pET-22b(+), pET-23abcd(+), pET-24abcd(+), pET-25b(+), pET-26b(+), pET-27b(+), pET-28abc(+), pET-29abc(+), pET-30abc(+), pET-31b(+), pET-32abc(+), pET-33b(+), pBAC-1, pBACgus-1, pBAC4x-1, pBACgus4x-1, pBAC-3cp, pBACgus-2cp, pBACsurf-1, plg, Signal plg, pYX, Selecta Vecta-Neo, Selecta Vecta - Hyg, and Selecta Vecta - Gpt from Novagen; pLexA, pB42AD, pGBT9, pAS2-1,



pGAD424, pACT2, pGAD GL, pGAD GH, pGAD10, pGilda, pEZM3, pEGFP, pEGFP-1, pEGFP-N, pEGFP-C, pEBFP, pGFPuv, pGFP, p6xHis-GFP, pSEAP2-Basic, pSEAP2-Contral, pSEAP2-Promoter, pSEAP2-Enhancer, p $\beta$ gal-Basic, p $\beta$ gal-Control, p $\beta$ gal-Promoter, p $\beta$ gal-Enhancer, pCMV $\beta$ , pTet-Off, pTet-On, pTK-Hyg, pRetro-Off, pRetro-On, pIRES1neo, pIRES1hyg, pLXSN, pLNCX, pLAPSN, pMAMneo, pMAMneo-CAT, pMAMneo-LUC, pPUR, pSV2neo, pYEX4T-1/2/3, pYEX-S1, pBacPAK-His, pBacPAK8/9, pAcUW31, BacPAK6, pTriplEx,  $\lambda$ gt10,  $\lambda$ gt11, pWE15, and  $\lambda$ TriplEx from Clontech; Lambda ZAP II, pBK-CMV, pBK-RSV, pBluescript II KS +/-, pBluescript II SK +/-, pAD-GAL4, pBD-GAL4 Cam, pSurfscrip, Lambda FIX II, Lambda DASH, Lambda EMBL3, Lambda EMBL4, SuperCos, pCR-Script Amp, pCR-Script Cam, pCR-Script Direct, pBS +/-, pBC KS +/-, pBC SK +/-, Phagescript, pCAL-n-EK, pCAL-n, pCAL-c, pCAL-kc, pET-3abcd, pET-11abcd, pSPUTK, pESP-1, pCMVLacI, pOPRSVI/MCS, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pMC1neo Poly A, pOG44, pOG45, pFRT $\beta$ GAL, pNEO $\beta$ GAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, and pRS416 from Stratagene.

Two-hybrid and reverse two-hybrid vectors of particular interest include pPC86, pDBLeu, pDBTrp, pPC97, p2.5, pGAD1-3, pGAD10, pACT, pACT2, pGADGL, pGADGH, pAS2-1, pGAD424, pGBT8, pGBT9, pGAD-GAL4, pLexA, pBD-GAL4, pHISi, pHISi-1, placZi, pB42AD, pDG202, pJK202, pJG4-5, pNLexA, pYESTrp and variants or derivatives thereof.

Yeast Expression Vectors of particular interest include pESP-1, pESP-2, pESC-His, pESC-Trp, pESC-URA, pESC-Leu (Stratagene), pRS401, pRS402, pRS411, pRS412, pRS421, pRS422, and variants or derivatives thereof.

According to the invention, the vectors comprising one or more nucleic acid molecules encoding one or more recombination sites, or mutants, variants, fragments, or derivatives thereof, may be produced by one of ordinary skill in the art without resorting to undue experimentation using standard molecular biology methods. For example, the vectors of the invention may be produced by introducing one or more of the nucleic acid molecules encoding one or more recombination sites (or mutants, fragments, variants or derivatives thereof) into one or more of the vectors described herein, according to the methods described,

for example, in Maniatis *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982). In a related aspect of the invention, the vectors may be engineered to contain, in addition to one or more nucleic acid molecules encoding one or more recombination sites (or portions thereof), one or more additional physical or functional nucleotide sequences, such as those encoding one or more multiple cloning sites, one or more transcription termination sites, one or more transcriptional regulatory sequences (e.g., one or more promoters, enhancers, or repressors), one or more selection markers or modules, one or more genes or portions of genes encoding a protein or polypeptide of interest, one or more translational signal sequences, one or more nucleotide sequences encoding a fusion partner protein or peptide (e.g., GST, His<sub>6</sub> or thioredoxin), one or more origins of replication, and one or more 5' or 3' polynucleotide tails (particularly a poly-G tail). According to this aspect of the invention, the one or more recombination site nucleotide sequences (or portions thereof) may optionally be operably linked to the one or more additional physical or functional nucleotide sequences described herein.

Preferred vectors according to this aspect of the invention include, but are not limited to: pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), pENTR3C (Figures 12A and 12B), pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), pENTR6 (Figures 15A and 15B), pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), pENTR9 (Figures 18A and 18B), pENTR10 (Figures 19A and 19B), pENTR11 (Figures 20A and 20B), pDEST1 (Figures 21A-D), pDEST2 (Figure 22A-D), pDEST3 (Figure 23A-D), pDEST4 (Figure 24A-D), pDEST5 (Figure 25A-D), pDEST6 (Figure 26A-D), pDEST7 (Figure 27A-C), pDEST8 (Figure 28A-D), pDEST9 (Figure 29A-E), pDEST10 (Figure 30A-D), pDEST11 (Figure 31A-D), pDEST12.2 (also known as pDEST12) (Figure 32A-D), pDEST13 (Figure 33A-C), pDEST14 (Figure 34A-D), pDEST15 (Figure 35A-D), pDEST16 (Figure 36A-D), pDEST17 (Figure 37A-D), pDEST18 (Figure 38A-D), pDEST19 (Figure 39A-D), pDEST20 (Figure 40A-D), pDEST21 (Figure 41A-E), pDEST22 (Figure 42A-D), pDEST23 (Figure 43A-D), pDEST24 (Figure 44A-D), pDEST25 (Figure 45A-D), pDEST26 (Figure 46A-D), pDEST27 (Figure 47A-D), pEXP501 (also known

as pCMVSPORT6) (Figure 48A-B), pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector) (Figure 49), pDONR202 (Figure 50), pDONR203 (also known as pEZ15812) (Figure 51), pDONR204 (Figure 52), pDONR205 (Figure 53), pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector) (Figure 54), pMAB58 (Figure 87), pMAB62 (Figure 88), pDEST28 (Figure 90), pDEST29 (Figure 91), pDEST30 (Figure 92), pDEST31 (Figure 93), pDEST32 (Figure 94), pDEST33 (Figure 95), pDEST34 (Figure 96), pDONR207 (Figure 97), pMAB85 (Figure 98), pMAB86 (Figure 99), and fragments, mutants, variants, and derivatives thereof. However, it will be understood by one of ordinary skill that the present invention also encompasses other vectors not specifically designated herein, which comprise one or more of the isolated nucleic acid molecules of the invention encoding one or more recombination sites or portions thereof (or mutants, fragments, variants or derivatives thereof), and which may further comprise one or more additional physical or functional nucleotide sequences described herein which may optionally be operably linked to the one or more nucleic acid molecules encoding one or more recombination sites or portions thereof. Such additional vectors may be produced by one of ordinary skill according to the guidance provided in the present specification.

### ***Polymerases***

Preferred polypeptides having reverse transcriptase activity (*i.e.*, those polypeptides able to catalyze the synthesis of a DNA molecule from an RNA template) for use in accordance with the present invention include, but are not limited to Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, Myeloblastosis Associated Virus (MAV) reverse transcriptase, Human Immunodeficiency Virus (HIV) reverse transcriptase, retroviral reverse transcriptase, retrotransposon reverse transcriptase, hepatitis B reverse transcriptase, cauliflower mosaic virus reverse transcriptase and bacterial reverse transcriptase. Particularly preferred are those polypeptides having reverse

transcriptase activity that are also substantially reduced in RNase H activity (*i.e.*, "RNase H" polypeptides). By a polypeptide that is "substantially reduced in RNase H activity" is meant that the polypeptide has less than about 20%, more preferably less than about 15%, 10% or 5%, and most preferably less than about 2%, of the RNase H activity of a wildtype or RNase H<sup>-</sup> enzyme such as wildtype M-MLV reverse transcriptase. The RNase H activity may be determined by a variety of assays, such as those described, for example, in U.S. Patent No. 5,244,797, in Kotewicz, M.L. *et al.*, *Nucl. Acids Res.* 16:265 (1988) and in Gerard, G.F., *et al.*, *FOCUS* 14(5):91 (1992), the disclosures of all of which are fully incorporated herein by reference. Suitable RNase H<sup>-</sup> polypeptides for use in the present invention include, but are not limited to, M-MLV H<sup>-</sup> reverse transcriptase, RSV H<sup>-</sup> reverse transcriptase, AMV H<sup>-</sup> reverse transcriptase, RAV H<sup>-</sup> reverse transcriptase, MAV H<sup>-</sup> reverse transcriptase, HIV H<sup>-</sup> reverse transcriptase, THERMOSCRIPT<sup>TM</sup> reverse transcriptase and THERMOSCRIPT<sup>TM</sup> II reverse transcriptase, and SUPERScript<sup>TM</sup> I reverse transcriptase and SUPERScript<sup>TM</sup> II reverse transcriptase, which are obtainable, for example, from Life Technologies, Inc. (Rockville, Maryland). See generally published PCT application WO 98/47912.

Other polypeptides having nucleic acid polymerase activity suitable for use in the present methods include thermophilic DNA polymerases such as DNA polymerase I, DNA polymerase III, Klenow fragment, T7 polymerase, and T5 polymerase, and thermostable DNA polymerases including, but not limited to, *Thermus thermophilus* (*Tth*) DNA polymerase, *Thermus aquaticus* (*Taq*) DNA polymerase, *Thermotoga neopolitana* (*Tne*) DNA polymerase, *Thermotoga maritima* (*Tma*) DNA polymerase, *Thermococcus litoralis* (*Tli* or VENT®) DNA polymerase, *Pyrococcus furiosus* (*Pfu*) DNA polymerase, *Pyrococcus* species GB-D (or DEEPVENT®) DNA polymerase, *Pyrococcus woosii* (*Pwo*) DNA polymerase, *Bacillus stearothermophilus* (*Bst*) DNA polymerase, *Sulfolobus acidocaldarius* (*Sac*) DNA polymerase, *Thermoplasma acidophilum* (*Tac*) DNA polymerase, *Thermus flavus* (*Tfl/Tub*) DNA polymerase, *Thermus ruber* (*Tru*) DNA polymerase, *Thermus brockianus* (DYNAZYME®) DNA polymerase, *Methanobacterium thermoautotrophicum* (*Mth*) DNA polymerase, and mutants,

variants and derivatives thereof. Such polypeptides are available commercially, for example from Life Technologies, Inc. (Rockville, MD), New England BioLabs (Beverly, MA), and Sigma/Aldrich (St. Louis, MO).

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### *Host Cells*

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The invention also relates to host cells comprising one or more of the nucleic acid molecules or vectors of the invention, particularly those nucleic acid molecules and vectors described in detail herein. Representative host cells that may be used according to this aspect of the invention include, but are not limited to, bacterial cells, yeast cells, plant cells and animal cells. Preferred bacterial host cells include *Escherichia* spp. cells (particularly *E. coli* cells and most particularly *E. coli* strains DH10B, Stbl2, DH5 $\alpha$ , DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY $\circledR$  DB3.1<sup>TM</sup> Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety), *Bacillus* spp. cells (particularly *B. subtilis* and *B. megaterium* cells), *Streptomyces* spp. cells, *Erwinia* spp. cells, *Klebsiella* spp. cells, *Serratia* spp. cells (particularly *S. marcessans* cells), *Pseudomonas* spp. cells (particularly *P. aeruginosa* cells), and *Salmonella* spp. cells (particularly *S. typhimurium* and *S. typhi* cells). Preferred animal host cells include insect cells (most particularly *Drosophila melanogaster* cells, *Spodoptera frugiperda* Sf9 and Sf21 cells and *Trichoplusia* High-Five cells), nematode cells (particularly *C. elegans* cells), avian cells, amphibian cells (particularly *Xenopus laevis* cells), reptilian cells, and mammalian cells (most particularly CHO, COS, VERO, BHK and human cells). Preferred yeast host cells include *Saccharomyces cerevisiae* cells and *Pichia pastoris* cells. These and other suitable host cells are available commercially, for example from Life Technologies, Inc. (Rockville, Maryland), American Type Culture Collection (Manassas, Virginia), and Agricultural Research Culture Collection (NRRL; Peoria, Illinois).

Methods for introducing the nucleic acid molecules and/or vectors of the invention into the host cells described herein, to produce host cells comprising one or more of the nucleic acid molecules and/or vectors of the invention, will be



familiar to those of ordinary skill in the art. For instance, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells using well known techniques of infection, transduction, transfection, and transformation. The nucleic acid molecules and/or vectors of the invention may be introduced alone or in conjunction with other the nucleic acid molecules and/or vectors. Alternatively, the nucleic acid molecules and/or vectors of the invention may be introduced into host cells as a precipitate, such as a calcium phosphate precipitate, or in a complex with a lipid. Electroporation also may be used to introduce the nucleic acid molecules and/or vectors of the invention into a host. Likewise, such molecules may be introduced into chemically competent cells such as *E. coli*. If the vector is a virus, it may be packaged *in vitro* or introduced into a packaging cell and the packaged virus may be transduced into cells. Hence, a wide variety of techniques suitable for introducing the nucleic acid molecules and/or vectors of the invention into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length, for example, in Sambrook, J., *et al.*, *Molecular Cloning, a Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp. 16.30-16.55 (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., pp. 213-234 (1992), and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987), which are illustrative of the many laboratory manuals that detail these techniques and which are incorporated by reference herein in their entireties for their relevant disclosures.

### ***Polypeptides***

In another aspect, the invention relates to polypeptides encoded by the nucleic acid molecules of the invention (including polypeptides and amino acid sequences encoded by all possible reading frames of the nucleic acid molecules of the invention), and to methods of producing such polypeptides. Polypeptides of the present invention include purified or isolated natural products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, insect, mammalian, avian and higher plant cells.



The polypeptides of the invention may be produced by synthetic organic chemistry, and are preferably produced by standard recombinant methods, employing one or more of the host cells of the invention comprising the vectors or isolated nucleic acid molecules of the invention. According to the invention, polypeptides are produced by cultivating the host cells of the invention (which comprise one or more of the nucleic acid molecules of the invention, preferably contained within an Expression Vector) under conditions favoring the expression of the nucleotide sequence contained on the nucleic acid molecule of the invention, such that the polypeptide encoded by the nucleic acid molecule of the invention is produced by the host cell. As used herein, "conditions favoring the expression of the nucleotide sequence" or "conditions favoring the production of a polypeptide" include optimal physical (*e.g.*, temperature, humidity, etc.) and nutritional (*e.g.*, culture medium, ionic) conditions required for production of a recombinant polypeptide by a given host cell. Such optimal conditions for a variety of host cells, including prokaryotic (bacterial), mammalian, insect, yeast, and plant cells will be familiar to one of ordinary skill in the art, and may be found, for example, in Sambrook, J., *et al.*, *Molecular Cloning, A Laboratory Manual, 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, (1989), Watson, J.D., *et al.*, *Recombinant DNA, 2nd Ed.*, New York: W.H. Freeman and Co., and Winnacker, E.-L., *From Genes to Clones*, New York: VCH Publishers (1987).

In some aspects, it may be desirable to isolate or purify the polypeptides of the invention (*e.g.*, for production of antibodies as described below), resulting in the production of the polypeptides of the invention in isolated form. The polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods of protein purification that are routine in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For example, His6 or GST fusion tags on polypeptides made by the methods of the invention may be isolated using appropriate affinity chromatography matrices which bind polypeptides bearing

His6 or GST tags, as will be familiar to one of ordinary skill in the art. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Isolated polypeptides of the invention include those comprising the amino acid sequences encoded by one or more of the reading frames of the polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or nucleotide sequences complementary thereto), or fragments, variants, mutants and derivatives thereof; the complete amino acid sequences encoded by the polynucleotides contained in the deposited clones described herein; the amino acid sequences encoded by polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences of the invention as set forth in Figure 9 (or a nucleotide sequence complementary thereto); or a peptide or polypeptide comprising a portion or a fragment of the above polypeptides. The invention also relates to additional polypeptides having one or more additional amino acids linked (typically by peptidyl bonds to form a nascent polypeptide) to the polypeptides encoded by the recombination site nucleotide sequences or the deposited clones. Such additional amino acid residues may comprise one or more functional peptide sequences, for example one or more fusion partner peptides (*e.g.*, GST, His<sub>6</sub>, Trx, etc.) and the like.

As used herein, the terms "protein," "peptide," "oligopeptide" and "polypeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of two or more amino acids, preferably five or more amino acids, or more preferably ten

or more amino acids, coupled by (a) peptidyl linkage(s), unless otherwise defined in the specific contexts below. As is commonly recognized in the art, all polypeptide formulas or sequences herein are written from left to right and in the direction from amino terminus to carboxy terminus.

5 It will be recognized by those of ordinary skill in the art that some amino acid sequences of the polypeptides of the invention can be varied without significant effect on the structure or function of the polypeptides. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine structure and activity. In general, it is possible to replace residues which form the tertiary structure, provided that  
10 residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the polypeptide.

Thus, the invention further includes variants of the polypeptides of the invention, including allelic variants, which show substantial structural homology  
15 to the polypeptides described herein, or which include specific regions of these polypeptides such as the portions discussed below. Such mutants may include deletions, insertions, inversions, repeats, and type substitutions (for example, substituting one hydrophilic residue for another, but not strongly hydrophilic for strongly hydrophobic as a rule). Small changes or such "neutral" or "conservative"  
20 amino acid substitutions will generally have little effect on activity.

Typical conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxylated residues Ser and Thr; exchange of the acidic residues Asp and Glu;  
25 substitution between the amidated residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr.

Thus, the fragment, derivative or analog of the polypeptides of the invention, such as those comprising peptides encoded by the recombination site nucleotide sequences described herein, may be (i) one in which one or more of the amino acid  
30 residues are substituted with a conservative or non-conservative amino acid residue (preferably a conservative amino acid residue), and such substituted amino acid residue may be encoded by the genetic code or may be an amino acid (e.g.,

desmosine, citrulline, ornithine, etc.) that is not encoded by the genetic code; (ii) one in which one or more of the amino acid residues includes a substituent group (e.g., a phosphate, hydroxyl, sulfate or other group) in addition to the normal "R" group of the amino acid; (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which additional amino acids are fused to the mature polypeptide, such as an immunoglobulin Fc region peptide, a leader or secretory sequence, a sequence which is employed for purification of the mature polypeptide (such as GST) or a proprotein sequence. Such fragments, derivatives and analogs are intended to be encompassed by the present invention, and are within the scope of those skilled in the art from the teachings herein and the state of the art at the time of invention.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Recombinantly produced versions of the polypeptides of the invention can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). As used herein, the term "substantially purified" means a preparation of an individual polypeptide of the invention wherein at least 50%, preferably at least 60%, 70%, or 75% and more preferably at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% (by mass) of contaminating proteins (*i.e.*, those that are not the individual polypeptides described herein or fragments, variants, mutants or derivatives thereof) have been removed from the preparation.

The polypeptides of the present invention include those which are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the polypeptides described herein. For example, preferred *attB1*-containing polypeptides of the invention include those that are at least about 50% identical, at least 60% identical, at least 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical,

to the polypeptide(s) encoded by the three reading frames of a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto), to a polypeptide encoded by a polynucleotide contained in the deposited cDNA clones described herein, or to a polypeptide encoded by a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising a nucleotide sequence of *attB1* having a nucleic acid sequence as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Analogous polypeptides may be prepared that are at least about 65% identical, more preferably at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identical, to the *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* polypeptides of the invention as depicted in Figure 9. The present polypeptides also include portions or fragments of the above-described polypeptides with at least 5, 10, 15, 20, or 25 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 65% "identical" to a reference amino acid sequence of a given polypeptide of the invention is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to 35 amino acid alterations per each 100 amino acids of the reference amino acid sequence of a given polypeptide of the invention. In other words, to obtain a polypeptide having an amino acid sequence at least 65% identical to a reference amino acid sequence, up to 35% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 35% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino (N-) or carboxy (C-) terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether a given amino acid sequence is, for example, at least 65% identical to the amino acid sequence of a given polypeptide of the invention can be determined



conventionally using known computer programs such as those described above for nucleic acid sequence identity determinations, or more preferably using the CLUSTAL W program (Thompson, J.D., *et al.*, *Nucleic Acids Res.* 22:4673-4680 (1994)).

5       The polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. In addition, as described in detail below, the polypeptides of the present invention can be used to raise polyclonal and monoclonal antibodies which are useful in a variety of assays for detecting  
10       protein expression, localization, detection of interactions with other molecules, or for the isolation of a polypeptide (including a fusion polypeptide) of the invention.

      In another aspect, the present invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention, which may be used to raise antibodies, particularly monoclonal antibodies, that bind  
15       specifically to a one or more of the polypeptides of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule.  
20       On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes (*see, e.g.*, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983)).

      As to the selection of peptides or polypeptides bearing an antigenic epitope  
25       (*i.e.*, that contain a region of a protein molecule to which an antibody can bind), it is well-known in the art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein (*see, e.g.*, Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)). Peptides capable of eliciting protein-reactive sera are  
30       frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are not confined to the immunodominant regions of intact proteins (*i.e.*, immunogenic epitopes) or to the amino or carboxy



termini. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing antibodies that bind to the mimicked protein; longer peptides, especially those containing proline residues, usually are effective (Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983)).

5        Epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least five, more preferably at least seven or more amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a given polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues and highly hydrophobic sequences are preferably avoided); sequences containing proline residues are particularly preferred.

10        Non-limiting examples of epitope-bearing polypeptides or peptides that can be used to generate antibodies specific for the polypeptides of the invention include certain epitope-bearing regions of the polypeptides comprising amino acid sequences encoded by polynucleotides comprising one or more of the recombination site-encoding nucleic acid molecules of the invention, including those encoding *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1* and *attR2* having the nucleotide sequences set forth in Figure 9 (or a nucleotide sequence complementary thereto); the complete amino acid sequences encoded by the three reading frames of the polynucleotides contained in the deposited clones described herein; and the amino acid sequences encoded by all reading frames of polynucleotides which hybridize under stringent hybridization conditions to polynucleotides having the nucleotide sequences encoding the recombination site sequences (or portions thereof) of the invention as set forth in Figure 9 (or a nucleic acid sequence complementary thereto). Other epitope-bearing polypeptides or peptides that may be used to generate antibodies specific for the polypeptides

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of the invention will be apparent to one of ordinary skill in the art based on the primary amino acid sequences of the polypeptides of the invention described herein, via the construction of Kyte-Doolittle hydrophilicity and Jameson-Wolf antigenic index plots of the polypeptides of the invention using, for example, PROTEAN computer software (DNASTAR, Inc.; Madison, Wisconsin).

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies. Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis (*see, e.g.*, U.S. Patent No. 4,631,211 and Houghten, R. A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985), both of which are incorporated by reference herein in their entireties).

As one of skill in the art will appreciate, the polypeptides of the present invention and epitope-bearing fragments thereof may be immobilized onto a solid support, by techniques that are well-known and routine in the art. By "solid support" is intended any solid support to which a peptide can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Linkage of the peptide of the invention to a solid support can be accomplished by attaching one or both ends of the peptide to the support. Attachment may also be made at one or more internal sites in the peptide. Multiple attachments (both internal and at the ends of the peptide) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments to the support, addition of an affinity tag sequence to the peptide can be used such as GST (Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Such affinity tags

may be used for the reversible attachment of the peptide to the support. Such immobilized polypeptides or fragments may be useful, for example, in isolating antibodies directed against one or more of the polypeptides of the invention, or other proteins or peptides that recognize other proteins or peptides that bind to one or more of the polypeptides of the invention, as described below.

As one of skill in the art will also appreciate, the polypeptides of the present invention and the epitope-bearing fragments thereof described herein can be combined with one or more fusion partner proteins or peptides, or portions thereof, including but not limited to GST, His<sub>6</sub>, Trx, and portions of the constant domain of immunoglobulins (Ig), resulting in chimeric or fusion polypeptides. These fusion polypeptides facilitate purification of the polypeptides of the invention (EP 0 394 827; Traunecker *et al.*, *Nature* 331:84-86 (1988)) for use in analytical or diagnostic (including high-throughput) format.

### Antibodies

In another aspect, the invention relates to antibodies that recognize and bind to the polypeptides (or epitope-bearing fragments thereof) or nucleic acid molecules (or portions thereof) of the invention. In a related aspect, the invention relates to antibodies that recognize and bind to one or more polypeptides encoded by all reading frames of one or more recombination site nucleic acid sequences or portions thereof, or to one or more nucleic acid molecules comprising one or more recombination site nucleic acid sequences or portions thereof, including but not limited to *att* sites (including *attB1*, *attB2*, *attP1*, *attP2*, *attL1*, *attL2*, *attR1*, *attR2* and the like), *lox* sites (*e.g.*, *loxP*, *loxP511*, and the like), FRT, and the like, or mutants, fragments, variants and derivatives thereof. See generally U.S. Patent No. 5,888,732, which is incorporated herein by reference in its entirety. The antibodies of the present invention may be polyclonal or monoclonal, and may be prepared by any of a variety of methods and in a variety of species according to methods that are well-known in the art. See, for instance, U.S. Patent No. 5,587,287; Sutcliffe, J.G., *et al.*, *Science* 219:660-666 (1983); Wilson *et al.*, *Cell* 37: 767 (1984); and Bittle, F.J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985). Antibodies specific for any of the polypeptides or nucleic acid molecules described

herein, such as antibodies specifically binding to one or more of the polypeptides encoded by the recombination site nucleotide sequences, or one or more nucleic acid molecules, described herein or contained in the deposited clones, antibodies against fusion polypeptides (*e.g.*, binding to fusion polypeptides between one or more of the fusion partner proteins and one or more of the recombination site polypeptides of the invention, as described herein), and the like, can be raised against the intact polypeptides or polynucleotides of the invention or one or more antigenic polypeptide fragments thereof.

As used herein, the term "antibody" (Ab) may be used interchangeably with the terms "polyclonal antibody" or "monoclonal antibody" (mAb), except in specific contexts as described below. These terms, as used herein, are meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to a polypeptide or nucleic acid molecule of the invention or a portion thereof. It will therefore be appreciated that, in addition to the intact antibodies of the invention, Fab, F(ab')<sub>2</sub> and other fragments of the antibodies described herein, and other peptides and peptide fragments that bind one or more polypeptides or polynucleotides of the invention, are also encompassed within the scope of the invention. Such antibody fragments are typically produced by proteolytic cleavage of intact antibodies, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Antibody fragments, and peptides or peptide fragments, may also be produced through the application of recombinant DNA technology or through synthetic chemistry.

Epitope-bearing peptides and polypeptides, and nucleic acid molecules or portions thereof, of the invention may be used to induce antibodies according to methods well known in the art, as generally described herein (*see, e.g.*, Sutcliffe, *et al.*, *supra*; Wilson, *et al.*, *supra*; and Bittle, F. J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985)).

Polyclonal antibodies according to this aspect of the invention may be made by immunizing an animal with one or more of the polypeptides or nucleic acid molecules of the invention described herein or portions thereof according to standard techniques (*see, e.g.*, Harlow, E., and Lane, D., *Antibodies: A*

*Laboratory Manual*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1988); Kaufman, P.B., *et al.*, In: *Handbook of Molecular and Cellular Methods in Biology and Medicine*, Boca Raton, Florida: CRC Press, pp. 468-469 (1995)). For producing antibodies that recognize and bind to the polypeptides or nucleic acid molecules of the invention or portions thereof, animals may be immunized with free peptide or free nucleic acid molecules; however, antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as albumin, KLH, or tetanus toxoid (particularly for producing antibodies against the nucleic acid molecules of the invention or portions thereof; *see* Harlow and Lane, *supra*, at page 154), or to a solid phase carrier such as a latex or glass microbead. For instance, peptides containing cysteine may be coupled to carrier using a linker such as m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice may be immunized with either free (if the polypeptide immunogen is larger than about 25 amino acids in length) or carrier-coupled peptides or nucleic acid molecules, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg peptide, polynucleotide, or carrier protein, and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of antibody which can be detected, for example, by ELISA assay using free peptide or nucleic acid molecule adsorbed to a solid surface. In another approach, cells expressing one or more of the polypeptides or polynucleotides of the invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies, according to routine immunological methods. In yet another method, a preparation of one or more of the polypeptides or polynucleotides of the invention is prepared and purified as described herein, to render it substantially free of natural contaminants. Such a preparation may then be introduced into an animal in order to produce polyclonal antisera of greater specific activity. The titer of antibodies in serum from an immunized animal, regardless of the method of immunization used, may be increased by selection of anti-peptide or anti-polynucleotide antibodies, for



instance, by adsorption to the peptide or polynucleotide on a solid support and elution of the selected antibodies according to methods well known in the art.

In an alternative method, the antibodies of the present invention are monoclonal antibodies (or fragments thereof which bind to one or more of the polypeptides of the invention). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al.*, *Nature* 256:495 (1975); Köhler *et al.*, *Eur. J. Immunol.* 6:511 (1976); Köhler *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a polypeptide or polynucleotide of the invention (or a fragment thereof), or with a cell expressing a polypeptide or polynucleotide of the invention (or a fragment thereof). The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP<sub>2</sub>O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterol.* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding one or more of the polypeptides or nucleic acid molecules of the invention, or fragments thereof. Hence, the present invention also provides hybridoma cells and cell lines producing monoclonal antibodies of the invention, particularly that recognize and bind to one or more of the polypeptides or nucleic acid molecules of the invention.

Alternatively, additional antibodies capable of binding to one or more of the polypeptides of the invention, or fragments thereof, may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and that, therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, antibodies specific for one or more of the polypeptides or polynucleotides of the invention, prepared as described above, are used to immunize an animal, preferably a mouse. The splenocytes of such an



animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to an antibody specific for one or more of the polypeptides or polynucleotides of the invention can be blocked by polypeptides of the invention themselves. Such antibodies comprise anti-idiotypic antibodies to the antibodies recognizing one or more of the polypeptides or polynucleotides of the invention, and can be used to immunize an animal to induce formation of further antibodies specific for one or more of the polypeptides or polynucleotides of the invention.

For use, the antibodies of the invention may optionally be detectably labeled by covalent or non-covalent attachment of one or more labels, including but not limited to chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, or nuclear magnetic resonance contrast agents or other labels.

Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

Examples of suitable radioisotopic labels include  $^3\text{H}$ ,  $^{111}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{75}\text{Se}$ ,  $^{152}\text{Eu}$ ,  $^{90}\text{Y}$ ,  $^{67}\text{Cu}$ ,  $^{217}\text{Bi}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Pb}$ ,  $^{47}\text{Sc}$ ,  $^{109}\text{Pd}$ , etc.

$^{111}\text{In}$  is a preferred isotope where in vivo imaging is used since it avoids the problem of dehalogenation of the  $^{125}\text{I}$  or  $^{131}\text{I}$ -labeled monoclonal antibody by the liver. In addition, this radionuclide has a more favorable gamma emission energy for imaging (Perkins *et al.*, *Eur. J. Nucl. Med.* 10:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 28:281-287 (1987)). For example,  $^{111}\text{In}$  coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Tr}$ , and  $^{56}\text{Fe}$ .

Examples of suitable fluorescent labels include an  $^{152}\text{Eu}$  label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a

phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, a green fluorescent protein (GFP) label, and a fluorescamine label.

Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

5        Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

10       Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

15       Typical techniques for binding the above-described labels to the antibodies of the invention are provided by Kennedy *et al.*, *Clin. Chim. Acta* 70:1-31 (1976), and Schurs *et al.*, *Clin. Chim. Acta* 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

20       It will be appreciated by one of ordinary skill that the antibodies of the present invention may alternatively be coupled to a solid support, to facilitate, for example, chromatographic and other immunological procedures using such solid phase-immobilized antibodies. Included among such procedures are the use of the antibodies of the invention to isolate or purify polypeptides comprising one or more epitopes encoded by the nucleic acid molecules of the invention (which may be fusion polypeptides or other polypeptides of the invention described herein), or to isolate or purify polynucleotides comprising one or more recombination site sequences of the invention or portions thereof. Methods for isolation and purification of polypeptides (and, by analogy, polynucleotides) by affinity chromatography, for example using the antibodies of the invention coupled to a solid phase support, are well-known in the art and will be familiar to one of ordinary skill. The antibodies of the invention may also be used in other applications, for example to cross-link or couple two or more proteins, polypeptides, polynucleotides, or portions thereof into a structural and/or functional complex. In one such use, an antibody of the invention may have two

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or more distinct epitope-binding regions that may bind, for example, a first polypeptide (which may be a polypeptide of the invention) at one epitope-binding region on the antibody and a second polypeptide (which may be a polypeptide of the invention) at a second epitope-binding region on the antibody, thereby bringing the first and second polypeptides into close proximity to each other such that the first and second polypeptides are able to interact structurally and/or functionally (as, for example, linking an enzyme and its substrate to carry out enzymatic catalysis, or linking an effector molecule and its receptor to carry out or induce a specific binding of the effector molecule to the receptor or a response to the effector molecule mediated by the receptor). Additional applications for the antibodies of the invention include, for example, the preparation of large-scale arrays of the antibodies, polypeptides, or nucleic acid molecules of the invention, or portions thereof, on a solid support, for example to facilitate high-throughput screening of protein or RNA expression by host cells containing nucleic acid molecules of the invention (known in the art as "chip array" protocols; *see, e.g.*, U.S. Patent Nos. 5,856,101, 5,837,832, 5,770,456, 5,744,305, 5,631,734, and 5,593,839, which are directed to production and use of chip arrays of polypeptides (including antibodies) and polynucleotides, and the disclosures of which are incorporated herein by reference in their entireties). By "solid support" is intended any solid support to which an antibody can be immobilized. Such solid supports include, but are not limited to nitrocellulose, diazocellulose, glass, polystyrene, polyvinylchloride, polycarbonate, polypropylene, polyethylene, dextran, Sepharose, agar, starch, nylon, beads and microtitre plates. Preferred are beads made of glass, latex or a magnetic material. Linkage of an antibody of the invention to a solid support can be accomplished by attaching one or both ends of the antibody to the support. Attachment may also be made at one or more internal sites in the antibody. Multiple attachments (both internal and at the ends of the antibody) may also be used according to the invention. Attachment can be via an amino acid linkage group such as a primary amino group, a carboxyl group, or a sulfhydryl (SH) group or by chemical linkage groups such as with cyanogen bromide (CNBr) linkage through a spacer. For non-covalent attachments, addition of an affinity tag sequence to the peptide can be used such as GST

(Smith, D.B., and Johnson, K.S., *Gene* 67:31 (1988)), polyhistidines (Hochuli, E., *et al.*, *J. Chromatog.* 411:77 (1987)), or biotin. Alternatively, attachment can be accomplished using a ligand which binds the Fc region of the antibodies of the invention, *e.g.*, protein A or protein G. Such affinity tags may be used for the reversible attachment of the antibodies to the support. Peptides may also be recognized via specific ligand-receptor interactions or using phage display methodologies that will be familiar to the skilled artisan, for their ability to bind polypeptides of the invention or fragments thereof.

### Kits

In another aspect, the invention provides kits which may be used in producing the nucleic acid molecules, polypeptides, vectors, host cells, and antibodies, and in the recombinational cloning methods, of the invention. Kits according to this aspect of the invention may comprise one or more containers, which may contain one or more of the nucleic acid molecules, primers, polypeptides, vectors, host cells, or antibodies of the invention. In particular, a kit of the invention may comprise one or more components (or combinations thereof) selected from the group consisting of one or more recombination proteins (*e.g.*, Int) or auxiliary factors (*e.g.* IHF and/or Xis) or combinations thereof, one or more compositions comprising one or more recombination proteins or auxiliary factors or combinations thereof (for example, GATEWAY™ LR Clonase™ Enzyme Mix or GATEWAY™ BP Clonase™ Enzyme Mix) one or more Destination Vector molecules (including those described herein), one or more Entry Clone or Entry Vector molecules (including those described herein), one or more primer nucleic acid molecules (particularly those described herein), one or more host cells (*e.g.* competent cells, such as *E. coli* cells, yeast cells, animal cells (including mammalian cells, insect cells, nematode cells, avian cells, fish cells, etc.), plant cells, and most particularly *E. coli* DB3, DB3.1 (preferably *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells; Life Technologies, Inc., Rockville, MD), DB4 and DB5; see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, and the corresponding U.S. Utility Application No. \_\_\_\_\_ of Hartley *et al.*, entitled "Cells Resistant to Toxic Genes and Uses Thereof," filed

on even day herewith, the disclosures of which are incorporated by reference herein in its entirety), and the like. In related aspects, the kits of the invention may comprise one or more nucleic acid molecules encoding one or more recombination sites or portions thereof, such as one or more nucleic acid molecules comprising a nucleotide sequence encoding the one or more recombination sites (or portions thereof) of the invention, and particularly one or more of the nucleic acid molecules contained in the deposited clones described herein. Kits according to this aspect of the invention may also comprise one or more isolated nucleic acid molecules of the invention, one or more vectors of the invention, one or more primer nucleic acid molecules of the invention, and/or one or more antibodies of the invention. The kits of the invention may further comprise one or more additional containers containing one or more additional components useful in combination with the nucleic acid molecules, polypeptides, vectors, host cells, or antibodies of the invention, such as one or more buffers, one or more detergents, one or more polypeptides having nucleic acid polymerase activity, one or more polypeptides having reverse transcriptase activity, one or more transfection reagents, one or more nucleotides, and the like. Such kits may be used in any process advantageously using the nucleic acid molecules, primers, vectors, host cells, polypeptides, antibodies and other compositions of the invention, for example in methods of synthesizing nucleic acid molecules (*e.g.*, via amplification such as via PCR), in methods of cloning nucleic acid molecules (preferably via recombinational cloning as described herein), and the like.

#### ***Optimization of Recombinational Cloning System***

The usefulness of a particular nucleic acid molecule, or vector comprising a nucleic acid molecule, of the invention in methods of recombinational cloning may be determined by any one of a number of assay methods. For example, Entry and Destination vectors of the present invention may be assessed for their ability to function (*i.e.*, to mediate the transfer of a nucleic acid molecule, DNA segment, gene, cDNA molecule or library from a cloning vector to an Expression Vector) by carrying out a recombinational cloning reaction as described in more detail in the Examples below and as described in U.S. Application Nos. 08/663,002, filed



June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of which are incorporated by reference herein in their entireties. Alternatively, the functionality of Entry and Destination Vectors prepared according to the invention may be assessed by examining the ability of these vectors to recombine and create cointegrate molecules, or to transfer a nucleic acid molecule of interest, using an assay such as that described in detail below in Example 19. Analogously, the formulation of compositions comprising one or more recombination proteins or combinations thereof, for example GATEWAY™ LR Clonase™ Enzyme Mix and GATEWAY™ BP Clonase™ Enzyme Mix, may be optimized using assays such as those described below in Example 18.

#### *Uses*

There are a number of applications for the compositions, methods and kits of the present invention. These uses include, but are not limited to, changing vectors, targeting gene products to intracellular locations, cleaving fusion tags from desired proteins, operably linking nucleic acid molecules of interest to regulatory genetic sequences (*e.g.*, promoters, enhancers, and the like), constructing genes for fusion proteins, changing copy number, changing replicons, cloning into phages, and cloning, *e.g.*, PCR products, genomic DNAs, and cDNAs. In addition, the nucleic acid molecules, vectors, and host cells of the invention may be used in the production of polypeptides encoded by the nucleic acid molecules, in the production of antibodies directed against such polypeptides, in recombinational cloning of desired nucleic acid sequences, and in other applications that may be enhanced or facilitated by the use of the nucleic acid molecules, vectors, and host cells of the invention.

In particular, the nucleic acid molecules, vectors, host cells, polypeptides, antibodies, and kits of the invention may be used in methods of transferring one or more desired nucleic acid molecules or DNA segments, for example one or more genes, cDNA molecules or cDNA libraries, into a cloning or Expression Vector for use in transforming additional host cells for use in cloning or



amplification of, or expression of the polypeptide encoded by, the desired nucleic acid molecule or DNA segment. Such recombinational cloning methods which may advantageously use the nucleic acid molecules, vectors, and host cells of the invention, are described in detail in the Examples below, and in commonly owned  
5 U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, 09/177,387, filed October 23, 1998, and 60/108,324, filed November 13, 1998, the disclosures of all of which are incorporated by reference herein in their entireties.

10 It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are readily apparent from the description of the invention contained herein in view of information known to the ordinarily skilled artisan, and may be made  
15 without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

## 20 *Examples*

### *Example 1: Recombination Reactions of Bacteriophage $\lambda$*

25 The *E. coli* bacteriophage  $\lambda$  can grow as a lytic phage, in which case the host cell is lysed, with the release of progeny virus. Alternatively, lambda can integrate into the genome of its host by a process called lysogenization (see Figure 60). In this lysogenic state, the phage genome can be transmitted to daughter cells for many generations, until conditions arise that trigger its excision from the genome.  
30 At this point, the virus enters the lytic part of its life cycle. The control of the switch between the lytic and lysogenic pathways is one of the best understood processes in molecular biology (M. Ptashne, *A Genetic Switch*, Cell Press, 1992).

The integrative and excisive recombination reactions of  $\lambda$ , performed *in vitro*, are the basis of Recombinational Cloning System of the present invention. They can be represented schematically as follows:



10                    The four att sites contain binding sites for the proteins that mediate the reactions. The wild type attP, attB, attL, and attR sites contain about 243, 25, 100, and 168 base pairs, respectively. The attB x attP reaction (hereinafter referred to as a "BP Reaction," or alternatively and equivalently as an "Entry Reaction" or a "Gateward Reaction") is mediated by the proteins Int and IHF. The attL x attR reaction (hereinafter referred to as an "LR Reaction," or alternatively and equivalently as a "Destination Reaction") is mediated by the proteins Int, IHF, and Xis. Int (integrase) and Xis (excisionase) are encoded by the  $\lambda$  genome, while IHF (integration host factor) is an *E. coli* protein. For a general review of lambda recombination, see: A. Landy, *Ann. Rev. Biochem.* 58: 913-949 (1989).

20                    **Example 2: Recombination Reactions of the Recombinational Cloning System**

The LR Reaction -- the exchange of a DNA segment from an Entry Clone to a Destination Vector -- is the *in vitro* version of the  $\lambda$  excision reaction:



30                    There is a practical imperative for this configuration: after an LR Reaction in one configuration of the present method, an att site usually separates a functional motif (such as a promoter or a fusion tag) from a nucleic acid molecule of interest in an Expression Clone, and the 25 bp attB site is much smaller than the attP, attL, and attR sites.

Note that the recombination reaction is conservative, i.e., there is no net synthesis or loss of base pairs. The DNA segments that flank the recombination

sites are merely switched. The wild type  $\lambda$  recombination sites are modified for purposes of the GATEWAY™ Cloning System, as follows:

To create certain preferred Destination Vectors, a part (43 bp) of attR was removed, to make the excisive reaction irreversible and more efficient (W. Bushman et al., *Science* 230: 906, 1985). The attR sites in preferred Destination Vectors of the invention are 125 bp in length. Mutations were made to the core regions of the att sites, for two reasons: (1) to eliminate stop codons, and (2) to ensure specificity of the recombination reactions (i.e., attR1 reacts only with attL1, attR2 reacts only with attL2, etc.).

Other mutations were introduced into the short (5 bp) regions flanking the 15 bp core regions of the attB sites to minimize secondary structure formation in single-stranded forms of attB plasmids, e.g., in phagemid ssDNA or in mRNA. Sequences of attB1 and attB2 to the left and right of a nucleic acid molecule of interest after it has been cloned into a Destination Vector are given in Figure 6.

Figure 61 illustrates how an Entry Clone and a Destination Vector recombine in the LR Reaction to form a co-integrate, which resolves through a second reaction into two daughter molecules. The two daughter molecules have the same general structure regardless of which pair of sites, attL1 and attR1 or attL2 and attR2, react first to form the co-integrate. The segments change partners by these reactions, regardless of whether the parental molecules are both circular, one is circular and one is linear, or both are linear. In this example, selection for ampicillin resistance carried on the Destination Vector, which also carries the death gene *ccdB*, provides the means for selecting only for the desired attB product plasmid.

### **Example 3: Protein Expression in the Recombinational Cloning System**

Proteins are expressed *in vivo* as a result of two processes, transcription (DNA into RNA), and translation (RNA into protein). For a review of protein expression in prokaryotes and eukaryotes, see Example 13 below. Many vectors (pUC, BlueScript, pGem) use interruption of a transcribed *lacZ* gene for blue-white screening. These plasmids, and many Expression Vectors, use the *lac* promoter to control expression of cloned genes. Transcription from the *lac*

promoter is turned on by adding the inducer IPTG. However, a low level of RNA is made in the absence of inducer, i.e., the lac promoter is never completely off. The result of this "leakiness" is that genes whose expression is harmful to *E. coli* may prove difficult or impossible to clone in vectors that contain the lac promoter, or they may be cloned only as inactive mutants.

In contrast to other gene expression systems, nucleic acid molecules cloned into an Entry Vector may be designed *not* to be expressed. The presence of the strong transcriptional terminator *rrnB* (Orosz, et al., *Eur. J. Biochem.* 201: 653, 1991) just upstream of the attL1 site keeps transcription from the vector promoters (drug resistance and replication origin) from reaching the cloned gene. However, if a toxic gene is cloned into a Destination Vector, the host may be sick, just as in other expression systems. But the reliability of subcloning by *in vitro* recombination makes it easier to recognize that this has happened -- and easier to try another expression option in accordance with the methods of the invention, if necessary.

#### **Example 4: Choosing the Right Entry Vector**

There are two kinds of choices that must be made in choosing the best Entry Vector, dictated by (1) the particular DNA segment that is to be cloned, and (2) what is to be accomplished with the cloned DNA segment. These factors are critical in the choice of Entry Vector used, because when the desired nucleic acid molecule of interest is moved from the Entry Vector to a Destination Vector, all the base pairs between the nucleic acid molecule of interest and the Int cutting sites in attL1 and attL2 (such as in Figure 6) move into the Destination Vector as well. For genomic DNAs that are not expressed as a result of moving into a Destination Vector, these decisions are not as critical.

For example, if an Entry Vector with certain translation start signals is used, those sequences will be translated into amino acids if an amino-terminal fusion to the desired nucleic acid molecule of interest is made. Whether the desired nucleic acid molecule of interest is to be expressed as fusion protein, native protein, or both, dictates whether translational start sequences must be included between the attB sites of the clone (native protein) or, alternatively, supplied by the Destination

Vector (fusion protein). In particular, Entry Clones that include translational start sequences may prove less suitable for making fusion proteins, as internal initiation of translation at these sites can decrease the yield of N-terminal fusion protein. These two types of expression afforded by the compositions and methods of the invention are illustrated in Figure 62.

No Entry Vector is likely to be optimal for all applications. The nucleic acid molecule of interest may be cloned into any of several optimal Entry Vectors.

As an example, consider pENTR7 (Figure 16) and pENTR11 (Figure 20), which are useful in a variety of applications, including (but not limited to):

- Cloning cDNAs from most of the commercially available libraries. The sites to the left and right of the *ccdB* death gene have been chosen so that directional cloning is possible if the DNA to be cloned does not have two or more of these restriction sites.

- Cloning of genes directionally: *Sall*, *Bam*HI, *Xmn*I (blunt), or *Kpn*I on the left of *ccdB*; *Not*I, *Xho*I, *Xba*I, or *Eco*RV (blunt), on the right.

- Cloning of genes or gene fragments with a blunt amino end at the *Xmn*I site. The *Xmn*I site has four of the six most favored bases for eukaryotic expression (see Example 13, below), so that if the first three bases of the DNA to be cloned are ATG, the open reading frame (ORF) will be expressed in eukaryotic cells (e.g., mammalian cells, insect cells, yeast cells) when it is transcribed in the appropriate Destination Vector. In addition, in pENTR11, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

- Cleaving off amino terminal fusions (e.g., His<sub>6</sub>, GST, or thioredoxin) using the highly specific TEV (Tobacco Etch Virus) protease (available from Life Technologies, Inc.). If the nucleic acid molecule of interest is cloned at the



blunt *Xmn*I site, TEV cleavage will leave two amino acids on the amino end of the expressed protein.

•Selecting against uncut or singly cut Entry Vector molecules during cloning with restriction enzymes and ligase. If the *ccdB* gene is not removed with a double digest, it will kill any recipient *E. coli* cell that does not contain a mutation that makes the cell resistant to *ccdB* (see U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, the disclosure of which is incorporated by reference herein in its entirety).

•Allowing production of amino fusions with ORFs in all cloning sites. There are no stop codons (in the attL1 reading frame) upstream of the *ccdB* gene.

In addition, pENTR11 is also useful in the following applications:

•Cloning cDNAs that have an *Nco*I site at the initiating ATG into the *Nco*I site. Similar to the *Xmn*I site, this site has four of the six most favored bases for eukaryotic expression. Also, a Shine-Dalgarno sequence is situated 8 bp upstream, for initiating protein synthesis in a prokaryotic host cell (particularly a bacterial cell, such as *E. coli*) at an ATG.

•Producing carboxy fusion proteins with ORFs positioned in phase with the reading frame convention for carboxy-terminal fusions (see Figure 20A).

Table 1 lists some non-limiting examples of Entry Vectors and their characteristics, and Figures 10-20 show their cloning sites. All of the Entry Vectors listed in Table 1 are available commercially from Life Technologies, Inc., Rockville, Maryland. Other Entry Vectors not specifically listed here, which comprise alternative or additional features may be made by one of ordinary skill using routine methods of molecular and cellular biology, in view of the disclosure contained herein.

Table 1 Examples of Entry Vectors

Designation	Mnemonic Name	Class of Entry Vector	Distinctive Cloning Sites	Amino Fusions	Native Protein in E.coli	Native Protein in Eukaryotic Cells	Protein Synthesis Features
pENTR-1A, 2B, 3C	Minimal blunt RF A, B, C	Alternative Reading Frame Vectors	Reading frame A, B, or C; blunt cut closest to attL1	Good	Poor	Good	Minimal amino acids between tag and protein; no SD
pENTR4	Minimal Nco	Restr. Enz. Cleavage Vectors	Nco I site (common in euk. cDNAs) closest to attL1	Good	Poor	Good	Good Kozac; no SD
pENTR5	Minimal Nde	Restr. Enz. Cleavage Vectors	NdeI site closest to attL1	Good	Poor	Poor at Nde I, Good at Xmn I	No SD; poor Kozac at Nde, good at Xmn
pENTR6	Minimal Sph	Restr. Enz. Cleavage Vectors	Sph I site closest to attL1	Good	Poor	Poor at Sph I, Good at Xmn I	No SD; poor Kozac at Sph, good at Xmn
pENTR7	TEV Blunt	TEV Cleavage Site Present	Xmn I (blunt) is first cloning site after TEV site	Good	Poor	Good at Xmn I site	TEV protease leaves Gly-Thr on amino end of protein; no SD
pENTR8	TEV Nco	TEV Cleavage Site Present	Nco I is first cloning site after TEV site	Good	Poor	Good	TEV protease leaves Gly-Thr on amino end of protein; no SD

pENTR9	TEV Nde	TEV Cleavage Site Present	Nde I is first cloning site after TEV site	Good	Poor	Poor	TEV protease leaves Gly-Thr on amino end of protein; no SD, poor Kozac
pENTR10	Nde with SD	Good SD for E.coli Expression	Strong SD; Nde I site, no TEV	Poor	Good	Poor	Strong SD, internal starts in amino fusions. Poor Kz. No TEV
pENTR11	2 X SD+Kozac	Good SD for E.coli Expression	Xmn I (blunt) and Nco I sites each preceded by SD and Kozac	Good	Good	Good	Strong SD/Koz Internal starts in amino fusions. No TEV

Entry vectors pENTR1A (Figures 10A and 10B), pENTR2B (Figures 11A and 11B), and pENTR3C (Figures 12A and 12B) are almost identical, except that the restriction sites are in different reading frames. Entry vectors pENTR4 (Figures 13A and 13B), pENTR5 (Figures 14A and 14B), and pENTR6 (Figures 15A and 15B) are essentially identical to pENTR1A, except that the blunt *DraI* site has been replaced with sites containing the ATG methionine codon: *NcoI* in pENTR4, *NdeI* in pENTR5, and *SphI* in pENTR6. Nucleic acid molecules that contain one of these sites at the initiating ATG can be conveniently cloned in these Entry vectors. The *NcoI* site in pENTR4 is especially useful for expression of nucleic acid molecules in eukaryotic cells, since it contains many of the bases that give efficient translation (see Example 13, below). (Nucleic acid molecules of interest cloned into the *NdeI* site of pENTR5 are not expected to be highly expressed in eukaryotic cells, because the cytosine at position -3 from the initiating ATG is rare in eukaryotic genes.)

Entry vectors pENTR7 (Figures 16A and 16B), pENTR8 (Figures 17A and 17B), and pENTR9 (Figures 18A and 18B) contain the recognition site for the TEV protease between the attL1 site and the cloning sites. Cleavage sites for *XmnI* (blunt), *NcoI*, and *NdeI*, respectively, are the most 5' sites in these Entry vectors. Amino fusions can be removed efficiently if nucleic acid molecules are cloned into these Entry vectors. TEV protease is highly active and highly specific.

#### ***Example 5: Controlling Reading Frame***

One of the trickiest tasks in expression of cloned nucleic acid molecules is making sure the reading frame is correct. (Reading frame is important if fusions are being made between two ORFs, for example between a nucleic acid molecule of interest and a His6 or GST domain.) For purposes of the present invention, the following convention has been adopted: The reading frame of the DNA cloned into any Entry Vector must be in phase with that of the attB1 site shown in Figure 16A, pENTR7. Notice that the six As of the attL1 site are split into two lysine codons (aaa aaa). The Destination Vectors that make amino fusions were constructed such that they enter the attR1 site in this reading frame.

Destination Vectors for carboxy terminal fusions were also constructed, including those containing His<sub>6</sub> (pDEST23; Figure 43), GST (pDEST24; Figure 44), or thioredoxin (pDEST25; Figure 45) C-terminal fusion sequences.

Therefore, if a nucleic acid molecule of interest is cloned into an Entry Vector so that the aaa aaa reading frame within the attL1 site is in phase with the nucleic acid molecule's ORF, amino terminal fusions will automatically be correctly phased, for all the fusion tags. This is a significant improvement over the usual case, where each different vector can have different restriction sites and different reading frames.

See Example 15 for a practical example of how to choose the most appropriate combinations of Entry Vector and Destination Vector.

#### Materials

Unless otherwise indicated, the following materials were used in the remaining Examples included herein:

#### 5X LR Reaction Buffer:

200-250 mM (preferably 250 mM) Tris-HCl, pH 7.5

250-350 mM (preferably 320 mM) NaCl

1.25-5 mM (preferably 4.75 mM) EDTA

12.5-35 mM (preferably 22-35 mM, and most preferably 35 mM)

Spermidine-HCl

1 mg/ml bovine serum albumin

#### GATEWAY™ LR Clonase™ Enzyme Mix:

per 4 µl of 1X LR Reaction Buffer:

150 ng carboxy-His<sub>6</sub>-tagged Int (see U.S. Appl. Nos. 60/108,324, filed November 13, 1998, and 09/438,358, filed November 12, 1999, both entirely incorporated by reference herein)



25 ng carboxy-His6-tagged Xis (see U.S. Appl. Nos. 60/108,324, filed  
November 13, 1998, and 09/438,358, filed November 12,  
1999, both entirely incorporated by reference herein)

30 ng IHF

50% glycerol

**5X BP Reaction Buffer:**

125 mM Tris-HCl, pH 7.5

110 mM NaCl

25 mM EDTA

25 mM Spermidine-HCl

5 mg/ml bovine serum albumin

**GATEWAY™ BP Clonase™ Enzyme Mix:**

per 4 µl of 1X BP Reaction Buffer:

200 ng carboxy-His6-tagged Int (see U.S. Appl. Nos. 60/108,324, filed  
November 13, 1998, and 09/438,358, filed November 12,  
1999, both entirely incorporated by reference herein)

80 ng IHF

50% glycerol

**10X Clonase Stop Solution:**

50 mM Tris-HCl, pH 8.0

1 mM EDTA

2 mg/ml Proteinase K

***Example 6: LR ("Destination") Reaction***

To create a new Expression Clone containing the nucleic acid molecule of  
interest (and which may be introduced into a host cell, ultimately for production  
of the polypeptide encoded by the nucleic acid molecule), an Entry Clone or  
Vector containing the nucleic acid molecule of interest, prepared as described.

herein, is reacted with a Destination Vector. In the present example, a  $\beta$ -Gal gene flanked by attL sites is transferred from an Entry Clone to a Destination Vector.

Materials needed:

- 5 X LR Reaction buffer
- Destination Vector (preferably linearized), 75-150 ng/ $\mu$ l
- Entry Clone containing nucleic acid molecule of interest, 100-300 ng in  $\leq 8 \mu$ l TE buffer
- Positive control Entry Clone (pENTR- $\beta$ -Gal) DNA (See note, below)
- Positive control Destination Vector, pDEST1 (pTrc), 75 ng/ $\mu$ l
- GATEWAY™ LR Clonase™ Enzyme Mix (stored at - 80° C)
- 10X Clonase Stop solution
- pUC19 DNA, 10 pg/ $\mu$ l
- Chemically competent *E. coli* cells (competence:  $\geq 1 \times 10^7$  CFU/ $\mu$ g), 400  $\mu$ l.
- LB Plates containing ampicillin (100  $\mu$ g/ml) and methicillin (200  $\mu$ g/ml)  $\pm$  X-gal and IPTG (See below)

Notes:

Preparation of the Entry Clone DNA: Miniprep DNA that has been treated with RNase works well. A reasonably accurate quantitation ( $\pm 50\%$ ) of the DNA to be cloned is advised, as the GATEWAY™ reaction appears to have an optimum of about 100-300 ng of Entry Clone per 20  $\mu$ l of reaction mix.

The positive control Entry Clone, pENTR- $\beta$ -Gal, permits functional analysis of clones based on the numbers of expected blue vs. white colonies on LB plates containing IPTG + Blue-gal (or X-gal), in addition to ampicillin (100  $\mu$ g/ml) and methicillin (200  $\mu$ g/ml). Because  $\beta$ -Galactosidase is a large protein, it often yields a less prominent band than many smaller proteins do on SDS protein gels.

In the Positive Control Entry Vector pENTR- $\beta$ -Gal, the coding sequence of  $\beta$ -Gal has been cloned into pENTR11 (Figures 20A and 20B), with translational start signals permitting expression in *E. coli*, as well as in eukaryotic

cells. The positive control Destination Vector, for example pDEST1 (Figure 21), is preferably linearized.

To prepare X-gal + IPTG plates, either of the following protocols may be used:

5

A. With a glass rod, spread over the surface of an LB agar plate: 40  $\mu$ l of 20 mg/ml X-gal (or Bluo-gal) in DMF plus 4  $\mu$ l 200 mg/ml IPTG. Allow liquid to adsorb into agar for 3-4 hours at 37° C before plating cells.

10

B. To liquid LB agar at ~45°C, add: X-gal (or Bluo-Gal) (20 mg/ml in DMF) to make 50  $\mu$ g/ml and IPTG (200 mM in water) to make 0.5-1 mM, just prior to pouring plates. Store X-gal and Bluo-Gal in a light-shielded container.

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Colony color may be enhanced by placing the plates at 5°C for a few hours after the overnight incubation at 37°C. Protocol B can give more consistent colony color than A, but A is more convenient when selection plates are needed on short notice.

20

Recombination in Clonase reactions continues for many hours. While incubations of 45-60 minutes are usually sufficient, reactions with large DNAs, or in which both parental DNAs are supercoiled, or which will be transformed into cells of low competence, can be improved with longer incubation times, such as 2-24 hours at 25°C.

#### Procedure:

25

1. Assemble reactions as follows (combine all components at room temperature, except GATEWAY™ LR Clonase™ Enzyme Mix ("Clonase LR"), before removing Clonase LR from frozen storage):

Component	Tube 1	Tube 2	Tube 3	Tube 4
	Neg.	Pos.	Neg.	Test
p-Gate- $\beta$ Gal, (Positive control Entry Clone) 75 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l		
pDEST1 (Positive control Destination Vector), 75 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l		
Your Entry Clone (100-300 ng)			1 - 8 $\mu$ l	1 - 8 $\mu$ l
Destination Vector for your nucleic acid molecule, 75 ng/ $\mu$ l			4 $\mu$ l	4 $\mu$ l
5 X LR Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	8 $\mu$ l	4 $\mu$ l	To 20 $\mu$ l	To 16 $\mu$ l
GATEWAY™ LR Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 $\mu$ l	---	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

2. Remove the GATEWAY™ LR Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ LR Clonase™ Enzyme Mix to reactions #2 and #4;
4. Return GATEWAY™ LR Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.
6. Add 2  $\mu$ l Clonase Stop solution to all reactions. Incubate for 20 min at 37° C. (This step usually increases the total number of colonies obtained by 10-20 fold.)
7. Transform 2  $\mu$ l into 100  $\mu$ l competent *E. coli*. Select on plates containing ampicillin at 100  $\mu$ g/ml.

#### **Example 7: Transformation of *E. coli***

To introduce cloning or Expression Vectors prepared using the recombinational cloning system of the invention, any standard *E. coli* transformation protocol should be satisfactory. The following steps are recommended for best results:

1. Let the mixture of competent cells and Recombinational Cloning System reaction product stand on ice at least 15 minutes prior to the heat-shock step. This gives time for the recombination proteins to dissociate from the DNA, and improves the transformation efficiency.

2. Expect the reaction to be about 1%-5% efficient, i.e., 2  $\mu$ l of the reaction should contain at least 100 pg of the Expression Clone plasmid (taking into account the amounts of each parental plasmid in the reaction, and the subsequent dilution). If the E. coli cells have a competence of  $10^7$  CFU/ $\mu$ g, 100 pg of the desired clone plasmid will give about 1000 colonies, or more, if the entire transformation is spread on one ampicillin plate.

3. Always do a control pUC DNA transformation. If the number of colonies is not what you expect, the pUC DNA transformation gives you an indication of where the problem was.

***Example 8: Preparation of attB-PCR Product***

For preparation of attB-PCR products in the PCR cloning methods described in Example 9 below, PCR primers containing attB1 and attB2 sequences are used. The attB1 and attB2 primer sequences are as follows:

**attB1:** 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCT-(template-specific sequence)-3'

**attB2:** 5'-GGGGACCACTTTGTACAAGAAAGCTGGGT-(template-specific sequence)-3'

The attB1 sequence should be added to the amino primer, and the attB2 sequence to the carboxy primer. The 4 guanines at the 5' ends of each of these primers enhance the efficiency of the minimal 25 bp attB sequences as substrates for use in the cloning methods of the invention.

Standard PCR conditions may be used to prepare the PCR product. The following suggested protocol employs PLATNUM *Taq* DNA Polymerase High

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Fidelity®, available commercially from Life Technologies, Inc. (Rockville, MD). This enzyme mix eliminates the need for hot starts, has improved fidelity over Taq, and permits synthesis of a wide range of amplicon sizes, from 200 bp to 10 kb, or more, even on genomic templates.

#### Materials needed:

- PLATINUM Taq DNA Polymerase High Fidelity® (Life Technologies, Inc.)
- attB1- and attB2- containing primer pair (see above) specific for your template
- DNA template (linearized plasmid or genomic DNA)
- 10X High Fidelity PCR Buffer
- 10 mM dNTP mix
- PEG/MgCl<sub>2</sub> Mix (30% PEG 8000, 30 mM MgCl<sub>2</sub>)

#### Procedure:

1.) Assemble the reaction as follows:

Component	Reaction with Plasmid Target	Reaction with <u>Genomic</u> Target
10X High Fidelity PCR Buffer	5 µl	5 µl
dNTP Mix 10 mM	1 µl	1 µl
MgSO <sub>4</sub> , 50mM	2 µl	2 µl
attB1 Primer, 10 µM	2 µl	1 µl
attB2 Primer, 10 µM	2 µl	1 µl
Template DNA	1-5 ng*	≥ 100 ng
PLATINUM Taq High Fidelity	2 µl	1 µl
Water	to 50 µl	to 50 µl

\* Use of higher amounts of plasmid template may permit fewer cycles (10-15) of PCR



2.) Add 2 drops mineral oil, as appropriate.

3.) Denature for 30 sec. at 94°C.

4.) Perform 25 cycles:

94°C for 15 sec-30 sec

55°C for 15 sec-30 sec

68°C for 1 min per kb of template.

5.) Following the PCR reaction, apply 1-2 µl of the reaction mixture to an agarose gel, together with size standards (e.g., 1 Kb Plus Ladder, Life Technologies, Inc.) and quantitation standards (e.g., Low Mass Ladder, Life Technologies, Inc.), to assess the yield and uniformity of the product.

Purification of the PCR product is recommended, to remove attB primer dimers which can clone efficiently into the Entry Vector. The following protocol is fast and will remove DNA <300 bp in size:

6.) Dilute the 50 µl PCR reaction to 200 µl with TE.

7.) Add 100 µl PEG/MgCl<sub>2</sub> Solution. Mix and centrifuge immediately at 13,000 RPM for 10 min at room temperature. Remove the supernatant (pellet is clear and hard to see).

8.) Dissolve the pellet in 50 µl TE and check recovery on a gel.

If the starting PCR template is a plasmid that contains the gene for Kan<sup>r</sup>, it is advisable to treat the completed PCR reaction with the restriction enzyme *DpnI*, to degrade the plasmid since unreacted residual starting plasmid is a potential source of false-positive colonies from the transformation of the GATEWAY™ Cloning System reaction. Adding ~5 units of *DpnI* to the completed PCR reaction and incubating for 15 min at 37°C will eliminate this potential problem. Heat inactivate the *DpnI* at 65°C for 15 min, prior to using the PCR product in the GATEWAY™ Cloning System reaction.

**Example 9: Cloning attB-PCR products into Entry Vectors via the BP ("Gateway") Reaction**

5 The addition of 5'-terminal attB sequences to PCR primers allows synthesis of a PCR product that is an efficient substrate for recombination with a Donor (attP) Plasmid in the presence of GATEWAY™ BP Clonase™ Enzyme Mix. This reaction produces an Entry Clone of the PCR product (See Figure 8).

10 The conditions of the Gateway Cloning reaction with an attB PCR substrate are similar to those of the BP Reaction (see Example 10 below), except that the attB-PCR product (see Example 8) substitutes for the Expression Clone, and the attB-PCR positive control (attB-tet<sup>r</sup>) substitutes for the Expression Clone Positive Control (GFP).

Materials needed:

- 15
- 5 X BP Reaction Buffer
  - Desired attB-PCR product DNA, 50-100 ng in  $\leq 8 \mu\text{l}$  TE.
  - Donor (attP) Plasmid (Figures 49-54), 75 ng/ $\mu\text{l}$ , supercoiled DNA
  - attB-tet<sup>r</sup> PCR product positive control, 25 ng/ $\mu\text{l}$
  - GATEWAY™ BP Clonase™ Enzyme Mix (stored at  $-80^{\circ}\text{C}$ )

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  - 10x Clonase Stop Solution
  - pUC19 DNA, 10 pg/ $\mu\text{l}$ .
  - Chemically competent E.coli cells (competence:  $\geq 1 \times 10^7$  CFU/ $\mu\text{g}$ ), 400  $\mu\text{l}$

Notes:

- 25
- Preparation of attB-PCR DNA: see Example 8.

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- The Positive Control attB-tet<sup>r</sup> PCR product contains a functional copy of the tet<sup>r</sup> gene of pBR322, with its own promoter. By plating the transformation of the control BP Reaction on kanamycin (50  $\mu\text{g/ml}$ ) plates (if kan<sup>r</sup> Donor Plasmids are used; see Figures 49-52) or an alternative selection agent (e.g., gentamycin, if gen<sup>r</sup> Donor Plasmids are used; see Figure 54), and then picking about 50 of these colonies onto plates with tetracycline (20  $\mu\text{g/ml}$ ), the

percentage of Entry Clones containing functional  $tet^r$  among the colonies from the positive control reaction can be determined (% Expression Clones = (number of  $tet^r$  +  $kan^r$  (or  $gen^r$ ) colonies/ $kan^r$  (or  $gen^r$ ) colonies).

5

**Procedure:**

1. Assemble reactions as follows. Combine all components except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from frozen storage.

10

Component	Neg.	Pos.	Test
	Tube 1	Tube 2	Tube 3
attB-PCR product, 50-100 ng			1 - 8 $\mu$ l
Donor (attP) Plasmid 75 ng/ $\mu$ l	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l
attB-PCR $tet^r$ control DNA (75 ng/ $\mu$ l)		4 $\mu$ l	
5 X BP Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	10 $\mu$ l	6 $\mu$ l	To 16 $\mu$ l
GATEWAY™ BP Clonase™ Enzyme Mix (store at -80° C, add last)	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

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2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80° C freezer, place immediately on ice. The Clonase takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes.

6. Add 2  $\mu$ l Proteinase K (2  $\mu$ g/ $\mu$ l) to all reactions. Incubate for 20 min at 37°C.
7. Transform 2  $\mu$ l into 100  $\mu$ l competent *E. coli*, as per 3.2, above. Select on LB plates containing kanamycin, 50  $\mu$ g/ml.

#### Results:

In initial experiments, primers for amplifying tetR and ampR from pBR322 were constructed containing only the tetR- or ampR-specific targeting sequences, the targeting sequences plus attB1 (for forward primers) or attB2 (for reverse primers) sequences shown in Figure 9, or the attB1 or attB2 sequences with a 5' tail of four guanines. The construction of these primers is depicted in Figure 65. After PCR amplification of tetR and ampR from pBR322 using these primers and cloning the PCR products into host cells using the recombinational cloning system of the invention, the results shown in Figure 66 were obtained. These results demonstrated that primers containing attB sequences provided for a somewhat higher number of colonies on the tetracycline and ampicillin plates. However, inclusion of the 5' extensions of four or five guanines on the primers in addition to the attB sequences provided significantly better cloning results, as shown in Figures 66 and 67. These results indicate that the optimal primers for cloning of PCR products using recombinational cloning will contain the recombination site sequences with a 5' extension of four or five guanine bases.

To determine the optimal stoichiometry between attB-containing PCR products and attP-containing Donor plasmid, experiments were conducted where the amount of PCR product and Donor plasmid were varied during the BP Reaction. Reaction mixtures were then transformed into host cells and plated on tetracycline plates as above. Results are shown in Figure 68. These results indicate that, for optimal recombinational cloning results with a PCR product in the size range of the tet gene, the amounts of attP-containing Donor plasmids are between about 100-500 ng (most preferably about 200-300 ng), while the optimal concentrations of attB-containing PCR products is about 25-100 ng (most preferably about 100 ng), per 20  $\mu$ l reaction.

Experiments were then conducted to examine the effect of PCR product size on efficiency of cloning via the recombinational cloning approach of the invention.

PCR products containing attB1 and attB2 sites, at sizes 256 bp, 1 kb, 1.4 kb, 3.4 kb, 4.6 kb, 6.9 kb and 10.1 kb were prepared and cloned into Entry vectors as described above, and host cells were transformed with the Entry vectors containing the cloned PCR products. For each PCR product, cloning efficiency was calculated relative to cloning of pUC19 positive control plasmids as follows:

$$\text{Cloning Efficiency} = \frac{\text{CFU/ng attB PCR product}}{\text{CFU/ng pUC19 control}} \times \frac{\text{Size (kb) PCR product}}{\text{Size (kb) pUC19 control}}$$

The results of these experiments are depicted in Figures 69A-69C (for 256 bp PCR fragments), 70A-70C (for 1 kb PCR fragments), 71A-71C (for 1.4 kb PCR fragments), 72A-72C (for 3.4 kb PCR fragments), 73A-73C (for 4.6 kb PCR fragments), 74 (for 6.9 kb PCR fragments), and 75-76 (for 10.1 kb PCR fragments). The results shown in these figures are summarized in Figure 77, for different weights and moles of input PCR DNA.

Together, these results demonstrate that attB-containing PCR products ranging in size from about 0.25 kb to about 5 kb clone relatively efficiently in the recombinational cloning system of the invention. While PCR products larger than about 5 kb clone less efficiently (apparently due to slow resolution of cointegrates), longer incubation times during the recombination reaction appears to improve the efficiency of cloning of these larger PCR fragments. Alternatively, it may also be possible to improve efficiency of cloning of large (> about 5 kb) PCR fragments by using lower levels of input attP Donor plasmid and perhaps attB-containing PCR product, and/or by adjusting reaction conditions (e.g., buffer conditions) to favor more rapid resolution of the cointegrates.

#### ***Example 10: The BP Reaction***

One purpose of the Gateward ("Entry") reaction is to convert an Expression Clone into an Entry Clone. This is useful when you have isolated an individual Expression Clone from an Expression Clone cDNA library, and you wish to transfer the nucleic acid molecule of interest into another Expression Vector, or

to move a population of molecules from an attB or attL library. Alternatively, you may have mutated an Expression Clone and now wish to transfer the mutated nucleic acid molecule of interest into one or more new Expression Vectors. In both cases, it is necessary first to convert the nucleic acid molecule of interest to an Entry Clone.

Materials needed:

- 5 X BP Reaction Buffer
- Expression Clone DNA, 100-300 ng in  $\leq 8 \mu\text{l}$  TE.
- Donor (attP) Vector, 75 ng/ $\mu\text{l}$ , supercoiled DNA
- Positive control attB-tet-PCR DNA, 25 ng/ $\mu\text{l}$
- GATEWAY™ BP Clonase™ Enzyme Mix (stored at  $-80^{\circ}\text{C}$ )
- Clonase Stop Solution (Proteinase K, 2  $\mu\text{g}/\mu\text{l}$ ).

Notes:

Preparation of the Expression Clone DNA: Miniprep DNA treated with RNase works well.

1. As with the LR Reaction (see Example 14), the BP Reaction is strongly influenced by the topology of the reacting DNAs. In general, the reaction is most efficient when one of the DNAs is linear and the other is supercoiled, compared to reactions where the DNAs are both linear or both supercoiled. Further, linearizing the attB Expression Clone (anywhere within the vector) will usually give more colonies than linearizing the Donor (attP) Plasmid. If finding a suitable cleavage site within your Expression Clone vector proves difficult, you may linearize the Donor (attP) Plasmid between the attP1 and attP2 sites (for example, at the *NcoI* site), avoiding the *ccdB* gene. Maps of Donor (attP) Plasmids are given in Figures 49-54.

Procedure:

1. Assemble reactions as follows. Combine all components at room temperature, except GATEWAY™ BP Clonase™ Enzyme Mix, before removing GATEWAY™ BP Clonase™ Enzyme Mix from freezer.



	Neg.	Pos.	Test
Component	Tube 1	Tube 2	Tube 3
Positive Control, attB-tet-PCR DNA, 25 ng/ $\mu$ l	4 $\mu$ l	4 $\mu$ l	
Desired attB Expression Clone DNA (100ng) linearized			1 - 8 $\mu$ l
Donor (attP) Plasmid, 75 ng/ $\mu$ l	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l
5 X BP Reaction Buffer	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
TE	10 $\mu$ l	6 $\mu$ l	To 16 $\mu$ l
GATEWAY™ BP Clonase™ Enzyme Mix (store at - 80° C, add last)	---	4 $\mu$ l	4 $\mu$ l
Total Volume	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l

2. Remove the GATEWAY™ BP Clonase™ Enzyme Mix from the -80°C freezer, place immediately on ice. The mixture takes only a few minutes to thaw.
3. Add 4  $\mu$ l of GATEWAY™ BP Clonase™ Enzyme Mix to the subcloning reaction, mix.
4. Return GATEWAY™ BP Clonase™ Enzyme Mix to - 80° C freezer.
5. Incubate tubes at 25° for at least 60 minutes. If both the attB and attP DNAs are supercoiled, incubation for 2-24 hours at 25°C is recommended.
6. Add 2  $\mu$ l Clonase Stop Solution. Incubate for 10 min at 37°C.
7. Transform 2  $\mu$ l into 100  $\mu$ l competent E. coli, as above. Select on LB plates containing 50  $\mu$ g/ml kanamycin.

***Example 11: Cloning PCR Products into Entry Vectors using Standard Cloning Methods***

**Preparation of Entry Vectors for Cloning of PCR Products**

All of the Entry Vectors of the invention contain the death gene ccdB as a stuffer between the "left" and "right" restriction sites. The advantage of this arrangement is that there is virtually no background from vector that has not been cut with both restriction enzymes, because the presence of the ccdB gene will kill

all standard E. coli strains. Thus it is necessary to cut each Entry Vector twice, to remove the ccdB fragment.

We strongly recommend that, after digestion of the Entry Vector with the second restriction enzyme, you treat the reaction with phosphatase (calf intestine alkaline phosphatase, CIAP or thermosensitive alkaline phosphatase, TSAP). The phosphatase can be added directly to the reaction mixture, incubated for an additional time, and inactivated. This step dephosphorylates both the vector and ccdB fragments, so that during subsequent ligation there is less competition between the ccdB fragment and the DNA of interest for the termini of the Entry Vector.

#### Blunt Cloning of PCR products

Generally PCR products do not have 5' phosphates (because the primers are usually 5' OH), and they are not necessarily blunt. (On this latter point, see Brownstein, et al., *BioTechniques* 20: 1006, 1996 for a discussion of how the sequence of the primers affects the addition of single 3' bases.) The following protocol repairs these two defects.

In a 0.5 ml tube, ethanol precipitate about 40 ng of PCR product (as judged from an agarose gel).

1. Dissolve the precipitated DNA in 10  $\mu$ l comprising 1  $\mu$ l 10 mM rATP, 1  $\mu$ l mixed 2 mM dNTPs (i.e., 2 mM each dATP, dCTP, dTTP, and dGTP), 2  $\mu$ l 5x T4 polynucleotide kinase buffer (350 mM Tris HCl (pH7.6), 50 mM  $MgCl_2$ , 500mM KCl, 5 mM 2-mercaptoethanol) 10 units T4 polynucleotide kinase, 1  $\mu$ l T4 DNA polymerase, and water to 10  $\mu$ l.
2. Incubate the tube at 37° for 10 minutes, then at 65° for 15 minutes, cool, centrifuge briefly to bring any condensate to the tip of the tube.
3. Add 5  $\mu$ l of the PEG/ $MgCl_2$  solution, mix and centrifuge at room temperature for 10 minutes. Discard supernatant.
4. Dissolve the invisible precipitate in 10  $\mu$ l containing 2  $\mu$ l 5x T4 DNA ligase buffer (Life Technologies, Inc.), 0.5 units T4 DNA ligase, and about 50 ng of blunt, phosphatase-treated Entry Vector.

5. Incubate at 25° for 1 hour, then 65° for 10 minutes. Add 90 µl TE, transform 10 µl into 50 - 100 µl competent *E. coli* cells.
6. Plate on kanamycin.

5 **Note:** In the above protocol, steps b-c simultaneously polish the ends of the PCR product (through the exonuclease and polymerase activities of T4 DNA polymerase) and phosphorylate the 5' ends (using T4 polynucleotide kinase). It is necessary to inactivate the kinase, so that the blunt, dephosphorylated vector in step e cannot self ligate. Step d (the PEG precipitation) removes all small  
10 molecules (primers, nucleotides), and has also been found to improve the yield of cloned PCR product by 50 fold.

#### Cloning PCR Products after Digestion with Restriction Enzymes

Efficient cloning of PCR products that have been digested with restriction  
15 enzymes includes three steps: inactivation of *Taq* DNA polymerase, efficient restriction enzyme cutting, and removal of small DNA fragments.

*Inactivation of Taq DNA Polymerase:* Carryover of *Taq* DNA polymerase and dNTPs into a RE digestion significantly reduces the success in cloning a PCR product (D. Fox et al., *FOCUS* 20(1):15, 1998), because *Taq* DNA polymerase  
20 can fill in sticky ends and add bases to blunt ends. Either TAQQUENCH™ (obtainable from Life Technologies, Inc.; Rockville, Maryland) or extraction with phenol can be used to inactivate the *Taq*.

*Efficient Restriction Enzyme Cutting:* Extra bases on the 5' end of each PCR primer help the RE cut near ends of PCR products. With the availability of  
25 cheap primers, adding 6 to 9 bases on the 5' sides of the restriction sites is a good investment to ensure that most of the ends are digested. Incubation of the DNA with a 5-fold excess of restriction enzyme for an hour or more helps ensure success.

*Removal of Small Molecules before Ligation:* Primers, nucleotides,  
30 primer dimers, and small fragments produced by the restriction enzyme digestion,

can all inhibit or compete with the desired ligation of the PCR product to the cloning vector. This protocol uses PEG precipitation to remove small molecules.

Protocol for cutting the ends of PCR products with restriction enzyme(s):

5

1. Inactivation of Taq DNA polymerase in the PCR product:

Option A: Extraction with Phenol

10

A1. Dilute the PCR reaction to 200  $\mu$ l with TE. Add an equal volume of phenol:chloroform:isoamyl alcohol, vortex vigorously for 20 seconds, and centrifuge for 1 minute at room temperature. Discard the lower phase.

15

A2. Extract the phenol from the DNA and concentrate as follows. Add an equal volume of 2-butanol (colored red with "Oil Red O" from Aldrich, if desired), vortex briefly, centrifuge briefly at room temperature. Discard the upper butanol phase. Repeat the extraction with 2-butanol. This time the volume of the lower aqueous phase should decrease significantly. Discard the upper 2-butanol phase.

20

A3. Ethanol precipitate the DNA from the aqueous phase of the above extractions. Dissolve in a 200  $\mu$ l of a suitable restriction enzyme (RE) buffer.

Option B: Inactivation with TaqQuench

25

B1. Ethanol precipitate an appropriate amount of PCR product (100 ng to 1  $\mu$ g), dissolve in 200  $\mu$ l of a suitable RE buffer.

B2. Add 2  $\mu$ l TaqQuench.

30

2. Add 10 to 50 units of restriction enzyme and incubate for at least 1 hour. Ethanol precipitate if necessary to change buffers for digestion at the other end of the PCR product.

3. Add ½ volume of the PEG/MgCl<sub>2</sub> mix to the RE digestion. Mix well and immediately centrifuge at room temperature for 10 minutes. Discard the supernatant (pellet is usually invisible), centrifuge again for a few seconds, discard any remaining supernatant.

5

4. Dissolve the DNA in a suitable volume of TE (depending on the amount of PCR product in the original amplification reaction) and apply an aliquot to an agarose gel to confirm recovery. Apply to the same gel 20-100 ng of the appropriate Entry Vector that will be used for the cloning.

10

***Example 12: Determining The Expected Size of the GATEWAY™ Cloning Reaction Products***

15 If you have access to a software program that will electronically cut and splice sequences, you can create electronic clones to aid you in predicting the sizes and restriction patterns of GATEWAY™ Cloning System recombination products.

20 The cleavage and ligation steps performed by the enzyme Int in the GATEWAY™ Cloning System recombination reactions mimic a restriction enzyme cleavage that creates a 7-bp 5'-end overhang followed by a ligation step that reseals the ends of the daughter molecules. The recombination proteins present in the Clonase cocktails (see Example 19 below) recognize the 15 bp core sequence present within all four types of att sites (in addition to other flanking sequences characteristic of each of the different types of att sites).

25 By treating these sites in your software program as if they were restriction sites, you can cut and splice your Entry Clones with various Destination Vectors and obtain accurate maps and sequences of the expected results from your GATEWAY™ Cloning System reactions.

30 ***Example 13: Protein Expression***

**Brief Review of Protein Expression**

*Transcription:* The most commonly used promoters in *E. coli* Expression Vectors are variants of the lac promoter, and these can be turned on by adding

IPTG to the growth medium. It is usually good to keep promoters off until expression is desired, so that the host cells are not made sick by the overabundance of some heterologous protein. This is reasonably easy in the case of the lac promoters used in *E. coli*. One needs to supply the *lac I* gene (or its more productive relative, the *lac I<sup>q</sup>* gene) to make *lac* repressor protein, which binds near the promoter and keeps transcription levels low. Some Destination Vectors for *E. coli* expression carry their own *lacI<sup>q</sup>* gene for this purpose. (However, lac promoters are always a little "on," even in the absence of IPTG.)

Controlling transcription in eukaryotic cells is not nearly so straightforward or efficient. The tetracycline system of Bujard and colleagues is the most successful approach, and one of the Destination Vectors (pDEST11; Figure 31) has been constructed to supply this function.

*Translation:* Ribosomes convert the information present in mRNA into protein. Ribosomes scan RNA molecules looking for methionine (AUG) codons, which begin nearly all nascent proteins. Ribosomes must, however, be able to distinguish between AUG codons that code for methionine in the middle of proteins from those at the start. Most often ribosomes choose AUGs that are 1) first in the RNA (toward the 5' end), and 2) have the proper sequence context. In *E. coli* the favored context (first recognized by Shine and Dalgarno, *Eur. J. Biochem.* 57: 221 (1975)) is a run of purines (As and Gs) from five to 12 bases upstream of the initiating AUG, especially AGGAGG or some variant.

In eukaryotes, a survey of translated mRNAs by Kozak (*J. Biol. Chem.* 266: 19867 (1991)) has revealed a preferred sequence context, gcc Acc ATGG, around the initiating methionine, with the A at -3 being most important, and a purine at +4 (where the A of the ATG is +1), preferably a G, being next most influential. Having an A at -3 is enough to make most ribosomes choose the first AUG of an mRNA, in plants, insects, yeast, and mammals. (For a review of initiation of protein synthesis in eukaryotic cells, see: Pain, V.M. *Eur. J. Biochem.* 236:747-771, 1996.)

*Consequences of Translation Signals for GATEWAY™ Cloning System:* First, translation signals (Shine-Dalgarno in *E. coli*, Kozak in eukaryotes) have to be close to the initiating ATG. The attB site is 25 base pairs long. Thus if



translation signals are desired near the natural ATG of the nucleic acid molecule of interest, they must be present in the Entry Clone of that nucleic acid molecule of interest. Also, when a nucleic acid molecule of interest is moved from an Entry Clone to a Destination vector, any translation signals will move along. The result is that the presence or absence of Shine-Dalgarno and/or Kozak sequences in the Entry Clone must be considered, with the eventual Destination Vectors to be used in mind.

Second, although ribosomes choose the 5' ATG most often, internal ATGs are also used to begin protein synthesis. The better the translation context around this internal ATG, the more internal translation initiation will be seen. This is important in the GATEWAY™ Cloning System, because you can make an Entry Clone of your nucleic acid molecule of interest, and arrange to have Shine-Dalgarno and/or Kozak sequences near the ATG. When this cassette is recombined into a Destination Vector that transcribes your nucleic acid molecule of interest, you get native protein. If you want, you can make a fusion protein in a different Destination Vector, since the Shine-Dalgarno and/or Kozak sequences do not contain any stop signals in the same reading frame. However, the presence of these internal translation signals may result in a significant amount of native protein being made, contaminating, and lowering the yield of, your fusion protein. This is especially likely with short fusion tags, like His6.

A good compromise can be recommended. If an Entry Vector like pENTR7 (Figure 16) or pENTR8 (Figure 17) is chosen, the Kozak bases are present for native eukaryotic expression. The context for *E. coli* translation is poor, so the yield of an amino-terminal fusion should be good, and the fusion protein can be digested with the TEV protease to make near-native protein following purification.

*Recommended Conditions for Synthesis of Proteins in E. coli:* When making proteins in *E. coli* it is advisable, at least initially, to incubate your cultures at 30°C, instead of at 37°C. Our experience indicates that proteins are less likely to form aggregates at 30°C. In addition, the yields of proteins from cells grown at 30°C frequently are improved.

The yields of proteins that are difficult to express may also be improved by inducing the cultures in mid-log phase of growth, using cultures begun in the morning from overnight growths, as opposed to harvesting directly from an overnight culture. In the latter case, the cells are preferably in late log or stationary growth, which can favor the formation of insoluble aggregates.

***Example 14: Constructing Destination Vectors from Existing Vectors***

Destination Vectors function because they have two recombination sites, attR1 and attR2, flanking a chloramphenicol resistance (CmR) gene and a death gene, ccdB. The GATEWAY™ Cloning System recombination reactions exchange the entire Cassette (except for a few bases comprising part of the attB sites) for the DNA segment of interest from the Entry Vector. Because attR1, CmR, ccdB gene, and attR2 are contiguous, they can be moved on a single DNA segment. If this Cassette is cloned into a plasmid, the plasmid becomes a Destination Vector. Figure 63 shows a schematic of the GATEWAY™ Cloning System Cassette; attR cassettes in all three reading frames contained in vectors pEZC15101, pEZC15102 and pEZC15103 are shown in Figures 64A, 64B, and 64C, respectively.

The protocol for constructing a Destination Vector is presented below. Keep in mind the following points:

- Destination Vectors must be constructed and propagated in one of the DB strains of *E. coli* (e.g., DB3.1, and particularly *E. coli* LIBRARY EFFICIENCY® DB3.1™ Competent Cells) available from Life Technologies, Inc. (and described in detail in U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), because the ccdB death gene will kill any *E. coli* strain that has not been mutated such that it will survive the presence of the ccdB gene.
- If your Destination Vector will be used to make a fusion protein, a GATEWAY™ Cloning System cassette with the correct reading frame must be used. The nucleotide sequences of the ends of the cassettes are shown in Figure 78. The reading frame of the fusion protein domain must

be in frame with the core region of the attR1 site (for an amino terminal fusion) so that the six As are translated into two lysine codons. For a C-terminal fusion protein, translation through the core region of the attR2 site should be in frame with -TAC-AAA-, to yield -Tyr-Lys-.

- Note that each reading frame Cassette has a different unique restriction site between the chloramphenicol resistance and ccdB genes (*Mlu*I for reading frame A, *Bgl*II for reading frame B, and *Xba*I for reading frame C; see Figure 63).
- Most standard vectors can be converted to Destination Vectors, by inserting the Entry Cassette into the MCS of that vector.

#### Protocol for Making a Destination Vector

1. If the vector will make an amino fusion protein, it is necessary to keep the "aaa aaa" triplets in attR1 in phase with the triplets of the fusion protein. Determine which Entry cassette to use as follows:

a.) Write out the nucleotide sequence of the existing vector near the restriction site into which the Entry cassette will be cloned. These must be written in triplets corresponding to the amino acid sequence of the fusion domain.

b.) Draw a vertical line through the sequence that corresponds to the restriction site end, after it has been cut and made blunt, i.e., after filling in a protruding 5' end or polishing a protruding 3' end.

c.) Choose the appropriate reading frame cassette:

- If the coding sequence of the blunt end ends after a complete codon triplet, use the reading frame A cassette. See Figures 78, 79 and 80.

- If the coding sequence of the blunt end ends in a single base, use the reading frame B cassette. See Figures 78, 79 and 81.

- If the coding sequence of the blunt end ends in two bases, use the reading frame C cassette. See Figures 78, 79, 82A-B, and 83A-C.

2. Cut one to five micrograms of the existing plasmid at the position where you wish your nucleic acid molecule of interest (flanked by att sites) to be after the recombination reactions. **Note:** it is better to remove as many of the MCS restriction sites as possible at this step. This makes it more likely that restriction enzyme sites within the GATEWAY™ Cloning System Cassette will be unique in the new plasmid, which is important for linearizing the Destination Vector (Example 14, below).

3. Remove the 5' phosphates with alkaline phosphatase. While this is not mandatory, it increases the probability of success.

4. Make the end(s) blunt with fill-in or polishing reactions. For example, to 1 µg of restriction enzyme-cut, ethanol-precipitated vector DNA, add:

- 20 µl 5x T4 DNA Polymerase Buffer (165 mM Tris-acetate (pH 7.9), 330 mM Na acetate, 50 mM Mg acetate, 500 µg/ml BSA, 2.5 mM DTT)
- 5 µl 10mM dNTP mix
- 1 Unit of T4 DNA Polymerase
- Water to a final volume of 100 µl
- Incubate for 15 min at 37°C.

5. Remove dNTPs and small DNA fragments: Ethanol precipitate (add three volumes of room temperature ethanol containing 0.1 M sodium acetate, mix well, immediately centrifuge at room temperature 5 - 10 minutes), dissolve wet precipitate in 200 µl TE, add 100 µl 30% PEG 8000, 30 mM MgCl<sub>2</sub>, mix well,

immediately centrifuge for 10 minutes at room temperature, discard supernatant, centrifuge again a few seconds, discard any residual liquid.

6. Dissolve the DNA to a final concentration of 10 - 50 ng per microliter. Apply 20 - 100 ng to a gel next to supercoiled plasmid and linear size standards to confirm cutting and recovery. The cutting does not have to be 100% complete, since you will be selecting for the chloramphenicol marker on the Entry cassette.

7. In a 10  $\mu$ l ligation reaction combine 10 - 50 ng vector, 10 - 20 ng of Entry Cassette (Figure 79), and 0.5 units T4 DNA ligase in ligase buffer. After one hour (or overnight, whichever is most convenient), transform 1  $\mu$ l into one of the DB strains of competent *E. coli* cells with a *gyrA462* mutation (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), preferably DB3.1, and most preferably *E. coli* LIBRARY EFFICIENCY@DB3.1™ Competent Cells. The *ccdB* gene on the Entry Cassette will kill other strains of *E. coli* that have not been mutated so as to survive the presence of the *ccdB* gene.

8. After expression in SOC medium, plate 10  $\mu$ l and 100  $\mu$ l on chloramphenicol-containing (30  $\mu$ g / ml) plates, incubate at 37° C.

9. Pick colonies, make miniprep DNA. Treat the miniprep with RNase A and store in TE. Cut with the appropriate restriction enzyme to determine the orientation of the Cassette. Choose clones with the attR1 site next to the amino end of the protein expression function of the plasmid.

#### Notes on Using Destination Vectors

- We have found that about ten-fold more colonies result from a GATEWAY™ Cloning System reaction if the Destination Vector is linear or relaxed. If the competent cells you use are highly competent ( $>10^8$  per microgram), linearizing the Destination Vector is less essential.

- The site or sites used for the linearization must be within the Entry Cassette. Sites that cut once or twice within each cassette are shown in Figures 80-82.
- Minipreps of Destination Vectors will work fine, so long as they have been treated with RNase. Since most DB strains are *endA*- (See U.S. Provisional Application No. 60/122,392, filed on March 2, 1999, which is incorporated herein by reference), minipreps can be digested with restriction enzymes without a prior phenol extraction.
- Reading the OD<sub>260</sub> of miniprep DNA is inaccurate unless the RNA and ribonucleotides have been removed, for example, by a PEG precipitation.

**Example 15: Some Options in Choosing Appropriate Entry Vectors and Destination Vectors: An Example**

In some applications, it may be desirable to express a nucleic acid molecule of interest in two forms: as an amino-terminal fusion in *E. coli*, and as a native protein in eukaryotic cells. This may be accomplished in any of several ways:

**Option 1:** Your choices depend on your nucleic acid molecule of interest and the fragment that contains it, as well as the available Entry Vectors. For eukaryotic translation, you need consensus bases according to Kozak (*J. Biol. Chem.* 266:19867, 1991) near the initiating methionine (ATG) codon. All of the Entry Vectors offer this motif upstream of the *XmnI* site (blunt cutter). One option is to amplify your nucleic acid molecule of interest, with its ATG, by PCR, making the amino end blunt and the carboxy end containing the natural stop codon followed by one of the "right side" restriction sites (*EcoRI*, *NotI*, *XhoI*, *EcoRV*, or *XbaI* of the pENTR vectors).

If you know your nucleic acid molecule of interest does not have, for example, an *XhoI* site, you can make a PCR product that has this structure:

Xho I

5' ATG nnn nnn --- nnn TAA ctc gag nnn nnn 3'  
 3' tac nnn nnn --- nnn att gag ctc nnn nnn 5'



After cutting with *Xho*I, the fragment is ready to clone:

5' ATG nnn nnn --- nnn TAA c 3'  
3' tac nnn nnn --- nnn att gag ct 5'

(If you follow this example, don't forget to put a phosphate on the amino oligo.)

**Option 2:** This PCR product could be cloned into two Entry Vectors to give the desired products, between the *Xmn*I and *Xho*I sites: pENTR1A (Figures 10A, 10B ) or pENTR7 (Figures 16A, 16B). If you clone into pENTR1A, amino fusions will have the minimal number of amino acids between the fusion domain and your nucleic acid molecule of interest, but the fusion cannot be removed with TEV protease. The converse is true of clones in pENTR7, i.e., an amino fusion can be cleaved with TEV protease, at the cost of more amino acids between the fusion and your nucleic acid molecule of interest.

In this example, let us choose to clone our hypothetical nucleic acid molecule of interest into pENTR7, between the *Xmn*I and *Xho*I sites. Once this is accomplished, several optional protocols using the Entry Clone pENTR7 may be followed:

**Option 3:** Since the nucleic acid molecule of interest has been amplified with PCR, it may be desirable to sequence it. To do this, transfer the nucleic acid molecule of interest from the Entry Vector into a vector that has priming sites for the standard sequencing primers. Such a vector is pDEST6 (Figures 26A, 26B). This Destination Vector places the nucleic acid molecule of interest in the opposite orientation to the lac promoter (which is leaky -- see Example 3 above). If the gene product is toxic to *E. coli*, this Destination Vector will minimize its toxicity.

**Option 4:** While the sequencing is going on, you might wish to check the expression of the nucleic acid molecule of interest in, for example, CHO cells, by recombining the nucleic acid molecule of interest into a CMV promoter vector (pDEST7, Figure 27; or pDEST12, Figure 32), or into a baculovirus vector (pDEST8, Figure 28; or pDEST10, Figure 30) for expression in insect cells. Both

of these vectors will transcribe the coding sequence of your nucleic acid molecule of interest, and translate it from the ATG of the PCR product using the Kozak bases upstream of the *XmnI* site.

**Option 5:** If you wish to purify protein, for example to make antibodies, you can clone the nucleic acid molecule of interest into a His6 fusion vector, pDEST2 (Figure 22). Since the nucleic acid molecule of interest is cloned downstream of the TEV protease cleavage domain of pENTR7 (Figure 16), the amino acid sequence of the protein produced will be:

[----- attB1 -----]      TEV protease

NH2- MSYYHHHHHHGITSLYKKAGFENLYFQ↓GTM----COOH

The attB site and the restriction sites used to make the Destination and Entry Vectors are translated into the underlined 11 amino acids (GITSLYKKAGF). Cleavage with TEV protease (arrow) leaves two amino acids, GT, on the amino end of the gene product.

See Figure 55 for an example of a nucleic acid molecule of interest, the chloramphenicol acetyl transferase (CAT) gene, cloned into pENTR7 (Figure 16) as a blunt (amino)-*XhoI* (carboxy) fragment, then cloned by recombination into the His6 fusion vector pDEST2 (Figure 22).

**Option 6:** If the His6 fusion protein is insoluble, you may go on and try a GST fusion. The appropriate Destination vector is pDEST3 (Figure 23).

**Option 7:** If you need to make RNA probes and prefer SP6 RNA polymerase, you can make the top strand RNA with your nucleic acid molecule of interest cloned into pSPORT+ (pDEST5 (Figures 25A, 25B)), and the bottom strand RNA with the nucleic acid molecule of interest cloned into pSPORT(-) (pDEST6 (Figures 26A, 26B)). Opposing promoters for T7 RNA polymerase and SP6 RNA polymerase are also present in these clones.

**Option 8:** It is often worthwhile to clone your nucleic acid molecule of interest into a variety of Destination Vectors in the same experiment. For example, if the number of colonies varies widely when the various recombination reactions are transformed into *E. coli*, this may be an indication that the nucleic acid molecule of interest is toxic in some contexts. (This problem is more clearly evident when a positive control gene is used for each Destination Vector.) Specifically, if many more colonies are obtained when the nucleic acid molecule of interest is recombined into pDEST6 than in pDEST5, there is a good chance that leakiness of the lac promoter is causing some expression of the nucleic acid molecule of interest in pSPORT "+" (which is not harmful in pDEST6 because the nucleic acid molecule of interest is in the opposite orientation).

***Example 16: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction***

In the BxP recombination (Entry or Gateward) reaction described herein, a DNA segment flanked by attB1 and attB2 sites in a plasmid conferring ampicillin resistance was transferred by recombination into an attP plasmid conferring kanamycin resistance, which resulted in a product molecule wherein the DNA segment was flanked by attL sites (attL1 and attL2). This product plasmid comprises an "attL Entry Clone" molecule, because it can react with a "attR Destination Vector" molecule via the LxR (Destination) reaction, resulting in the transfer of the DNA segment to a new (ampicillin resistant) vector. In the previously described examples, it was necessary to transform the BxP reaction products into *E. coli*, select kanamycin resistant colonies, grow those colonies in liquid culture, and prepare miniprep DNA, before reacting this DNA with a Destination Vector in an LxR reaction.

The goal of the following experiment was to eliminate the transformation and miniprep DNA steps, by adding the BxP Reaction products directly to an LxR Reaction. This is especially appropriate when the DNA segment flanked by attB sites is a PCR product instead of a plasmid, because the PCR product cannot give

ampicillin-resistant colonies upon transformation, whereas attB plasmids (in general) carry an ampicillin resistance gene. Thus use of a PCR product flanked by attB sites in a BxP Reaction allows one to select for the ampicillin resistance encoded by the desired attB product of a subsequent LxR Reaction.

Two reactions were prepared: Reaction A, negative control, no attB PCR product, (8  $\mu$ l) contained 50 ng pEZC7102 (attP Donor plasmid, confers kanamycin resistance) and 2  $\mu$ l BxP Clonase (22 ng /  $\mu$ l Int protein and 8 ng/ $\mu$ l IHF protein) in BxP buffer (25 mM Tris HCl, pH 7.8, 70 mM KCl, 5 mM spermidine, 0.5 mM EDTA, 250  $\mu$ g / ml BSA). Reaction B (24  $\mu$ l) contained 150 ng pEZC7102, 6  $\mu$ l BxP Clonase, and 120 ng of the attB -tet-PCR product in the same buffer as reaction A. The attB - tet - PCR product comprised the tetracycline resistance gene of plasmid pBR322, amplified with two primers containing either attB1 or attB2 sites, and having 4 Gs at their 5' ends, as described earlier.

The two reactions were incubated at 25°C for 30 minutes. Then aliquots of these reactions were added to new components that comprised LxR Reactions or appropriate controls for the LxR Reaction. Five new reactions were thus produced:

**Reaction 1:** 5  $\mu$ l of reaction A was added to a 5  $\mu$ l LxR Reaction containing 25 ng *Nco*I-cut pEZC8402 (the attR Destination Vector plasmid) in LxR buffer (37.5 mM Tris HCl, pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375  $\mu$ g / ml BSA), and 1  $\mu$ l of GATEWAY™ LR Clonase™ Enzyme Mix (total volume of 10  $\mu$ l).

**Reaction 2:** Same as reaction 1, except 5  $\mu$ l of reaction B (positive) were added instead of reaction A (negative).

**Reaction 3:** Same as reaction 2, except that the amounts of *Nco*-cut pEZC8402 and GATEWAY™ LR Clonase™ Enzyme Mix were doubled, to 50 ng and 2  $\mu$ l, respectively.

**Reaction 4:** Same as reaction 2, except that 25 ng of pEZ11104 (a positive control attL Entry Clone plasmid) were added in addition to the aliquot of reaction B.

**Reaction 5:** Positive control LxR Reaction, containing 25 ng *NcoI*-cut pEZC8402, 25 ng pEZ11104, 37.5 mM Tris HCl pH 7.7, 16.5 mM NaCl, 35 mM KCl, 5 mM spermidine, 375 µg / ml BSA and 1 µl GATEWAY™ LR Clonase™ Enzyme Mix in a total volume of 5 µl.

All five reactions were incubated at 25°C for 30 minutes. Then, 1 µl aliquots of each of the above five reactions, plus 1 µl from the remaining volume of Reaction B, the standard BxP Reaction, were used to transform 50 µl competent DH5α *E. coli*. DNA and cells were incubated on ice for 15 min., heat shocked at 42°C for 45 sec., and 450 µl SOC were added. Each tube was incubated with shaking at 37°C for 60 min. Aliquots of 100 µl and 400 µl of each transformation were plated on LB plates containing either 50 µg/ml kanamycin or 100 µg/ml ampicillin (see Table 2). A transformation with 10 pg of pUC19 DNA (plated on LB-amp<sub>100</sub>) served as a control on the transformation efficiency of the DH5α cells. Following incubation overnight at 37°C, the number of colonies on each plate was determined.

Results of these reactions are shown in Table 2.

**Table 2\***

Reaction No.:	1	2	3	4	5	6
	Number of Colonies					
Vol. plated:	Neg. Control BxP Reaction	1X pEZC8402 and LR Clonase™	2X pEZC8402 and LR Clonase™	LxR Reaction with Pos. Control DNA	LxR Reaction alone	BxP Reaction alone
100 µl	2	1	8	9	~1000	~1000
400 µl	5	10	35	62	>2000	>2000
Selection:	Kan	Amp	Amp	Amp	Amp	Kan

\*(Transformation with pUC 19 DNA yielded  $1.4 \times 10^9$  CFU/µg DNA.)

34 of the 43 colonies obtained from Reaction 3 were picked into 2 ml Terrific Broth with 100 µg/ml ampicillin and these cultures were grown overnight, with shaking, at 37°C. 27 of the 34 cultures gave at least moderate growth, and of these 24 were used to prepare miniprep DNA, using the standard protocol. These 24 DNAs were initially analyzed as supercoiled (SC) DNA on a 1% agarose gel to identify those with inserts and to estimate the sizes of the inserts. Fifteen of the 24 samples displayed SC DNA of the size predicted (5553 bp) if **tetx7102** had correctly recombined with **pEJC8402** to yield **tetx8402**. One of these samples contained two plasmids, one of ~5500 bp and a one of ~3500 bp. The majority of the remaining clones were approximately 4100 bp in size.

All 15 of the clones displaying SC DNA of predicted size (~5500 bp) were analyzed by two different double digests with restriction endonucleases to confirm the structure of the expected product: **tetx8402**. (See plasmid maps, Figures 57-59) In one set of digests, the DNAs were treated with Not I and Eco RI, which should cut the predicted product just outside both attB sites, releasing the tet<sup>r</sup> insert on a fragment of 1475 bp. In the second set of digests, the DNAs were digested with *NotI* and with *NruI*. *NruI* cleaves asymmetrically within the subcloned tet<sup>r</sup> insert, and together with *NotI* will release a fragment of 1019 bp.

Of the 15 clones analyzed by double restriction digestion, 14 revealed the predicted sizes of fragments for the expected product.

#### **Interpretation:**

The DNA components of Reaction B, pEJC7102 and attB-tet-PCR, are shown in Figure 56. The desired product of BxP Reaction B is **tetx7102**, depicted in Figure 57. The LxR Reaction recombines the product of the BxP Reaction, **tetx7102** (Figure 57), with the Destination Vector, pEJC8402, shown in Figure 58. The LxR Reaction with **tetx7102** plus pEJC8402 is predicted to yield the desired product **tetx8402**, shown in Figure 59.

Reaction 2, which combined the BxP Reaction and LxR Reaction, gave few colonies beyond those of the negative control Reaction. In contrast, Reaction 3, with twice the amount of pEJC8402 (Figure 58) and LxR Clonase, yielded a



larger number of colonies. These colonies were analyzed further, by restriction digestion, to confirm the presence of expected product. Reaction 4 included a known amount of attL Entry Clone plasmid in the combined BxP-plus-LxR reaction. But reaction 4 yielded only about 1% of the colonies obtained when the same DNA was used in a LxR reaction alone, Reaction 6. This result suggests that the LxR reaction may be inhibited by components of the BxP reaction.

Restriction endonuclease analysis of the products of Reaction 3 revealed that a sizeable proportion of the colonies (14 of the 34 analyzed) contained the desired *tet<sup>r</sup>* subclone, *tetx8402* (Figure 59).

The above results establish the feasibility of performing first a BxP recombination reaction followed by a LxR recombination reaction -- in the same tube -- simply by adding the appropriate buffer mix, recombination proteins, and DNAs to a completed BxP reaction. This method should prove useful as a faster method to convert attB-containing PCR products into different Expression Clones, eliminating the need to isolate first the intermediate attL-PCR insert subclones, before recombining these with Destination Vectors. This may prove especially valuable for automated applications of these reactions.

This same one-tube approach allows for the rapid transfer of nucleic acid molecules contained in attB plasmid clones into new functional vectors as well. As in the above examples, attL subclones generated in a BxP Reaction can be recombined directly with various Destination Vectors in a LxR reaction. The only additional requirement for using attB plasmids, instead of attB-containing PCR products, is that the Destination Vector(s) employed must contain a different selection marker from the one present on the attB plasmid itself and the attP vector.

Two alternative protocols for a one-tube reaction have also proven useful and somewhat more optimal than the conditions described above.

#### Alternative 1:

Reaction buffer contained 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.25 mM EDTA, 2.5 mM spermidine, and 200 µg/ml BSA. After a 16 (or 3) hour incubation of the PCR product (100 ng) + attP Donor plasmid (100 ng) +

GATEWAY™ BP Clonase™ Enzyme Mix + Destination Vector (100 ng), 2 µl of GATEWAY™ LR Clonase™ Enzyme Mix (per 10 µl reaction mix) was added and the mixture was incubated an additional 6 (or 2) hours at 25°C. Stop solution was then added as above and the mixture was incubated at 37°C as above and transformed by electroporation with 1 µl directly into electrocompetent host cells. Results of this series of experiments demonstrated that longer incubation times (16 hours vs. 3 hours for the BP Reaction, 6 hours vs. 2 hours for the LR Reaction) resulted in about twice as many colonies being obtained as for the shorter incubation times. With two independent genes, 10/10 colonies having the correct cloning patterns were obtained.

Alternative 2:

A standard BP Reaction under the reaction conditions described above for Alternative 1 was performed for 2 hours at 25°C. Following the BP Reaction, the following components were added to the reaction mixture in a total volume of 7 µl:

20 mM Tris-HCl, pH 7.5

100 mM NaCl

5 µg/ml Xis-His6

15% glycerol

~1000 ng of Destination Vector

The reaction mixture was then incubated for 2 hours at 25°C, and 2.5 µl of stop solution (containing 2 µg/ml proteinase K) was added and the mixture was incubated at 37°C for an additional 10 minutes. Chemically competent host cells were then transformed with 2 µl of the reaction mixture, or electrocompetent host cells (e.g., EMax DH10B cells; Life Technologies, Inc.) were electroporated with 2 µl of the reaction mixture per 25-40 µl of cells. Following transformation, mixtures were diluted with SOC, incubated at 37°C, and plated as described above on media selecting for the selection markers on the Destination Vector and the Entry clone (B x P reaction product). Analogous results to those described for Alternative 1 were obtained with these reaction conditions -- a higher level of colonies containing correctly recombined reaction products were observed.

***Example 17: Demonstration of a One-tube Transfer of a PCR Product (or Expression Clone) to Expression Clone via a Recombinational Cloning Reaction***

5           Single-tube transfer of PCR product DNA or Expression Clones into Expression Clones by recombinational cloning has also been accomplished using a procedure modified from that described in Example 16. This procedure is as follows:

10           •Perform a standard BP (Gateway) Reaction (see Examples 9 and 10) in 20 µl volume at 25°C for 1 hour.

15           •After the incubation is over, take a 10 µl aliquot from the 20 µl total volume and add 1 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes. This first aliquot can be used for transformation and gel assay of BP reaction analysis. Plate BP reaction transformation on LB plates with Kanamycin (50 µg/ml).

20           •Add the following reagents to the remaining 10 µl aliquot of the BP reaction:

1 µl of 0.75 M NaCl

2 µl of destination vector (150 ng/µl)

4 µl of LR Clonase™ (after thawing and brief mixing)

25           •Mix all reagents well and incubate at 25°C for 3 hours. Stop the reaction at the end of incubation with 1.7 µl of Proteinase K (2 mg/ml) and incubate at 37°C for 10 minutes.

30           •Transform 2 µl of the completed reaction into 100 µl of competent cells. Plate 100 µl and 400 µl on LB plates with Ampicillin (100 µg/ml).

**Notes:**

•If your competent cells are less than  $10^8$  CFU/µg, and you are concerned about getting enough colonies, you can improve the yield several fold by incubating the

BP reaction for 6-20 hours. Electroporation also can yield better colony output than chemical transformation.

•PCR products greater than about 5-6 kb show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using longer incubation times for both BP and LR steps.

•If you want to move your insert gene into several destination vectors simultaneously, then scale up the initial BP reaction volume so that you have a 10 µl aliquot for adding each destination vector.

***Example 18: Optimization of GATEWAY™ Clonase™ Enzyme Compositions***

The enzyme compositions containing Int and IHF (for BP Reactions) were optimized using a standard functional recombinational cloning reaction (a BP reaction) between attB-containing plasmids and attP-containing plasmids, according to the following protocol:

**Materials and Methods:**

***Substrates:***

AttP - supercoiled pDONR201

AttB - linear ~ 1Kb [<sup>3</sup>H]PCR product amplified from pEZC7501

***Proteins:***

IntH6 -- His<sub>6</sub>-carboxy- tagged λ Integrase

IHF -- Integration Host Factor

***Clonase:***

50 ng/µl IntH6 and 20 ng/µl IHF, admixed in 25 mM Tris- HCl (pH 7.5), 22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, and 50% glycerol.

*Reaction Mixture (total volume of 40  $\mu$ l):*

1000 ng AttP plasmid

600 ng AttB [ $^3$ H] PCR product

8  $\mu$ l Clonase (400 ng IntH6, 160 ng IHF) in 25 mM Tris-HCl (pH 7.5),  
22 mM NaCl, 5 mM EDTA, 1 mg/ml BSA, 5 mM Spermidine, 5 mM  
DTT.

Reaction mixture was incubated for 1 hour at 25°C, 4  $\mu$ l of 2  $\mu$ g/ $\mu$ l  
proteinase K was added and mixture was incubated for an additional 20 minutes  
at 37°C. Mixture was then extracted with an equal volume of Phenol/Chloroform/  
Isoamyl alcohol. The aqueous layer was then collected, and 0.1 volumes of 3 M  
sodium acetate and 2 volumes of cold 100% ethanol were added. Tubes were  
then spun in a microcentrifuge at maximum RPM for 10 minutes at room  
temperature. Ethanol was decanted, and pellets were rinsed with 70% ethanol and  
re-centrifuged as above. Ethanol was decanted, and pellets were allowed to air  
dry for 5-10 minutes and then dissolved in 20  $\mu$ l of 33 mM Tris-Acetate (pH 7.8),  
66 mM potassium acetate, 10 mM magnesium acetate, 1 mM DTT, and 1mM  
ATP. 2 units of exonuclease V (e.g., Plasmid Safe; EpiCentre, Inc., Madison, WI)  
was then added, and the mixture was incubated at 37°C for 30 minutes.

Samples were then TCA-washed by spotting 30  $\mu$ l of reaction mixture  
onto a Whatman GF/C filter, washing filters once with 10% TCA + 1% NaPPi for  
10 minutes, three times with 5% TCA for 5 minutes each, and twice with ethanol  
for 5 minutes each. Filters were then dried under a heat lamp, placed into a  
scintillation vial, and counted on a  $\beta$  liquid scintillation counter (LSC).

The principle behind this assay is that, after exonuclease V digestion, only  
double-stranded circular DNA survives in an acid-insoluble form. All DNA  
substrates and products that have free ends are digested to an acid-soluble form  
and are not retained on the filters. Therefore, only the  $^3$ H-labeled attB linear DNA  
which ends up in circular form after both inter- and intramolecular integration is  
complete is resistant to digestion and is recovered as acid-insoluble product.  
Optimal enzyme and buffer formulations in the Clonase compositions therefore are  
those that give the highest levels of circularized  $^3$ H-labeled attB-containing

sequences, as determined by highest cpm in the LSC. Although this assay was designed for optimization of GATEWAY™ BP Clonase™ Enzyme Mix compositions (Int + IHF), the same type of assay may be performed to optimize GATEWAY™ LR Clonase™ Enzyme Mix compositions (Int + IHF + Xis), except that the reaction mixtures would comprise 1000 ng of AttR (instead of AttP) and 600 ng of AttL (instead of AttB), and 40 ng of His<sub>6</sub>-carboxy- tagged Xis (XisH6) in addition to the IntH6 and IHF.

***Example 19: Testing Functionality of Entry and Destination Vectors***

As part of assessment of the functionality of particular vectors of the invention, it is important to functionally test the ability of the vectors to recombine. This assessment can be carried out by performing a recombinational cloning reaction (as schematized in Figures 2, 4, and 5A and 5B, and as described herein and in commonly owned U.S. Application Nos. 08/486,139, filed June 7, 1995, 08/663,002, filed June 7, 1996 (now U.S. Patent No. 5,888,732), 09/005,476, filed January 12, 1998, and 09/177,387, filed October 23, 1998, the disclosures of all of which are incorporated by reference herein in their entireties), by transforming E. coli and scoring colony forming units. However, an alternative assay may also be performed to allow faster, more simple assessment of the functionality of a given Entry or Destination Vector by agarose gel electrophoresis. The following is a description of such an in vitro assay.

**Materials and Methods:**

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type att site, were used for the generation of PCR products containing attL or attR sites, respectively. Plasmid templates were linearized with *A*hoNI, phenol extracted, ethanol precipitated and dissolved in TE to a concentration of 1 ng/μl.



PCR primers (capital letters represent base changes from wildtype):

attL1            gggg agcct gctttttGtacAaa gttggcatta taaaaaagca ttgc  
attL2            gggg agcct gctttCttGtacAaa gttggcatta taaaaaagca ttgc  
attL right        tgttgccggg aagctagagt aa

attR1            gggg Acaag ttTgtaCaaaaaagc tgaacgaga aacgtaaaat  
attR2            gggg Acaag ttTgtaCaaGaaagc tgaacgaga aacgtaaaat  
attR right        ca gacggcatga tgaacctgaa

PCR primers were dissolved in TE to a concentration of 500 pmol/ $\mu$ l. Primer mixes were prepared, consisting of attL1 + attLright primers, attL2 + attLright primers, attR1 + attRright primers, and attR2 + attRright primers, each mix containing 20 pmol/ $\mu$ l of each primer.

PCR reactions:

1  $\mu$ l plasmid template (1 ng)  
1  $\mu$ l primer pairs (20 pmoles of each)  
3  $\mu$ l of H<sub>2</sub>O  
45  $\mu$ l of Platinum PCR SuperMix® (Life Technologies, Inc.)

Cycling conditions (performed in MJ thermocycler):

95°C/2 minutes  
94°C/30 seconds  
25 cycles of 58°C/30 seconds and 72°C/1.5 minutes  
72°C/5 minutes  
5°C/hold

The resulting attL PCR product was 1.5 kb, and the resulting attR PCR product was 1.0 kb.

PCR reactions were PEG/MgCl<sub>2</sub> precipitated by adding 150  $\mu$ l H<sub>2</sub>O and 100  $\mu$ l of 3x PEG/ MgCl<sub>2</sub> solution followed by centrifugation. The PCR products were dissolved in 50  $\mu$ l of TE. Quantification of the PCR product was performed by gel electrophoresis of 1  $\mu$ l and was estimated to be 50-100 ng/ $\mu$ l.

Recombination reactions of PCR products containing attL or attR sites with GATEWAY™ plasmids was performed as follows:

8 µl of H<sub>2</sub>O

2 µl of attL or attR PCR product (100-200 ng)

5 2 µl of GATEWAY™ plasmid (100 ng)

4 µl of 5x Destination buffer

4 µl of GATEWAY™ LR Clonase™ Enzyme Mix

20 µl total volume (the reactions can be scaled down to a 5 µl total volume by adjusting the volumes of the components to about ¼ of those shown above, while keeping the stoichiometries the same).

10 Clonase reactions were incubated at 25°C for 2 hours. 2 µl of proteinase K (2 mg/ml) was added to stop the reaction. 10 µl was then run on a 1 % agarose gel. Positive control reactions were performed by reacting attL1 PCR product (1.0 kb) with attR1 PCR product (1.5 kb) and by similarly reacting attL2 PCR product with attR2 PCR product to observe the formation of a larger (2.5 kb) recombination product. Negative controls were similarly performed by reacting attL1 PCR product with attR2 PCR product and vice versa or reactions of attL PCR product with an attL plasmid, etc.

20 In alternative assays, to test attB Entry vectors, plasmids containing single attP sites were used. Plasmids containing single att sites could also be used as recombination substrates in general to test all Entry and Destination vectors (*i.e.*, those containing attL, attR, attB and attP sites). This would eliminate the need to do PCR reactions.

25 Results:

Destination and Entry plasmids when reacted with appropriate att-containing PCR products formed linear recombinant molecules that could be easily visualized on an agarose gel when compared to control reactions containing no attL or attR PCR product. Thus, the functionality of Destination and Entry vectors constructed according to the invention may be determined either by carrying out the Destination or Entry recombination reactions as depicted in

Figures 2, 4, and 5A and 5B, or more rapidly by carrying out the linearization assay described in this Example.

***Example 20: PCR Cloning Using Universal Adapter-Primers***

As described herein, the cloning of PCR products using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) requires the addition of attB sites (attB1 and attB2) to the ends of gene-specific primers used in the PCR reaction. The protocols described in the preceding Examples suggest that the user add 29 bp (25 bp containing the attB site plus four G residues) to the gene-specific primer. It would be advantageous to high volume users of the GATEWAY™ PCR Cloning System to generate attB-containing PCR product using universal attB adapter-primers in combination with shorter gene-specific primers containing a specified overlap to the adapters. The following experiments demonstrate the utility of this strategy using universal attB adapter-primers and gene-specific primers containing overlaps of various lengths from 6 bp to 18 bp. The results demonstrate that gene-specific primers with overlaps of 10 bp to 18 bp can be used successfully in PCR amplifications with universal attB adapter-primers to generate full-length PCR products. These PCR products can then be successfully cloned with high fidelity in a specified orientation using the GATEWAY™ PCR Cloning System.

**Methods and Results:**

To demonstrate that universal attB adapter-primers can be used with gene-specific primers containing partial attB sites in PCR reactions to generate full-length PCR product, a small 256 bp region of the human hemoglobin cDNA was chosen as a target so that intermediate sized products could be distinguished from full-length products by agarose gel electrophoresis.

The following oligonucleotides were used:

B1-Hgb: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T-5' -Hgb\*

B2-Hgb: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T-3' -Hgb\*\*

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18B1-Hgb: TG TAC AAA AAA GCA GGC T-5'-Hgb  
 18B2-Hgb: TG TAC AAG AAA GCT GGG T-3'-Hgb  
 15B1-Hgb: AC AAA AAA GCA GGC T-5'-Hgb  
 15B2-Hgb: AC AAG AAA GCT GGG T-3'-Hgb  
 5 12B1-Hgb: AA AAA GCA GGC T-5'-Hgb  
 12B2-Hgb: AG AAA GCT GGG T-3'-Hgb  
 11B1-Hgb: A AAA GCA GGC T-5'-Hgb  
 11B2-Hgb: G AAA GCT GGG T-3'-Hgb  
 10B1-Hgb: AAA GCA GGC T-5'-Hgb  
 10 10B2-Hgb: AAA GCT GGG T-3'-Hgb  
 9B1-Hgb: AA GCA GGC T-5'-Hgb  
 9B2-Hgb: AA GCT GGG T-3'-Hgb  
 8B1-Hgb: A GCA GGC T-5'-Hgb  
 8B2-Hgb: A GCT GGG T-3'-Hgb  
 15 7B1-Hgb: GCA GGC T-5'-Hgb  
 7B2-Hgb: GCT GGG T-3'-Hgb  
 6B1-Hgb: CA GGC T-5'-Hgb  
 6B2-Hgb: CT GGG T-3'-Hgb  
  
 20 attB1 adapter: GGGG ACA AGT TTG TAC AAA AAA GCA GGC T  
 attB2 adapter: GGGG ACC ACT TTG TAC AAG AAA GCT GGG T  
  
 \* -5'-Hgb = GTC ACT AGC CTG TGG AGC AAG A  
 \*\* -3'-Hgb = AGG ATG GCA GAG GGA GAC GAC A

25

The aim of these experiments was to develop a simple and efficient universal adapter PCR method to generate attB containing PCR products suitable for use in the GATEWAY™ PCR Cloning System. The reaction mixtures and thermocycling conditions should be simple and efficient so that the universal adapter PCR method could be routinely applicable to any PCR product cloning application.

30

PCR reaction conditions were initially found that could successfully amplify predominately full-length PCR product using gene-specific primers containing 18bp and 15 bp overlap with universal attB primers. These conditions are outlined below:

35

10 pmoles of gene-specific primers  
10 pmoles of universal attB adapter-primers  
1 ng of plasmid containing the human hemoglobin cDNA.  
100 ng of human leukocyte cDNA library DNA.  
5  $\mu$ l of 10x PLATINUM Taq HiFi® reaction buffer (Life Technologies, Inc.)  
2  $\mu$ l of 50 mM MgSO<sub>4</sub>  
1  $\mu$ l of 10 mM dNTPs  
0.2  $\mu$ l of PLATINUM Taq HiFi® (1.0 unit)  
H<sub>2</sub>O to 50  $\mu$ l total reaction volume

Cycling conditions:

25 x 

95°C/5 min
94°C/15 sec
50°C/30 sec
68°C/1 min
68°C/5 min
5°C/hold

To assess the efficiency of the method, 2  $\mu$ l (1/25) of the 50  $\mu$ l PCR reaction was electrophoresed in a 3 % Agarose-1000 gel. With overlaps of 12 bp or less, smaller intermediate products containing one or no universal attB adapter predominated the reactions. Further optimization of PCR reaction conditions was obtained by titrating the amounts of gene-specific primers and universal attB adapter-primers. The PCR reactions were set up as outlined above except that the amounts of primers added were:

0, 1, 3 or 10 pmoles of gene-specific primers  
0, 10, 30 or 100 pmoles of adapter-primers

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universal attB adapter-primers should generate a robust PCR reaction with a much higher overall yield of product.

PCR products from reactions using the 18 bp, 15 bp, 12 bp, 11 bp and 10 bp overlap gene-specific primers were purified using the CONCERT® Rapid PCR Purification System (PCR products greater than 500 bp can be PEG precipitated). The purified PCR products were subsequently cloned into an attP containing plasmid vector using the GATEWAY™ PCR Cloning System (Life Technologies, Inc.; Rockville, MD) and transformed into *E. coli*. Colonies were selected and counted on the appropriate antibiotic media and screened by PCR for correct inserts and orientation.

Raw PCR products (unpurified) from the attB adapter PCR of a plasmid clone of part of the human beta-globin (Hgb) gene were also used in GATEWAY™ PCR Cloning System reactions. PCR products generated with the full attB B1/B2-Hgb, the 12B1/B2, 11B1/B2 and 10B1/B2 attB overlap Hgb primers were successfully cloned into the GATEWAY™ pENTR21 attP vector (Figure 49). 24 colonies from each (24 x 4 = 96 total) were tested and each was verified by PCR to contain correct inserts. The cloning efficiency expressed as cfu/ml is shown below:

Primer Used	cfu/ml
Hgb full attB	8,700
Hgb 12 bp overlap	21,000
Hgb 11 bp overlap	20,500
Hgb 10 bp overlap	13,500
GFP control	1,300

Interestingly, the overlap PCR products cloned with higher efficiency than did the full attB PCR product. Presumably, and as verified by visualization on agarose gel, the adapter PCR products were slightly cleaner than was the full attB PCR product. The differences in colony output may also reflect the proportion of PCR product molecules with intact attB sites.

Using the attB adapter PCR method, PCR primers with 12 bp attB overlaps were used to amplify cDNAs of different sizes (ranging from 1 to 4 kb)

from a leukocyte cDNA library and from first strand cDNA prepared from HeLa total RNA. While three of the four cDNAs were able to be amplified by this method, a non-specific amplification product was also observed that under some conditions would interfere with the gene-specific amplification. This non-specific product was amplified in reactions containing the attB adapter-primers alone without any gene-specific overlap primers present. The non-specific amplification product was reduced by increasing the stringency of the PCR reaction and lowering the attB adapter PCR primer concentration.

These results indicate that the adapter-primer PCR approach described in this Example will work well for cloned genes. These results also demonstrate the development of a simple and efficient method to amplify PCR products that are compatible with the GATEWAY™ PCR Cloning System that allows the use of shorter gene-specific primers that partially overlap universal attB adapter-primers. In routine PCR cloning applications, the use of 12 bp overlaps is recommended. The methods described in this Example can thus reduce the length of gene-specific primers by up to 17 residues or more, resulting in a significant savings in oligonucleotide costs for high volume users of the GATEWAY™ PCR Cloning System. In addition, using the methods and assays described in this Example, one of ordinary skill can, using only routine experimentation, design and use analogous primer-adapters based on or containing other recombination sites or fragments thereof, such as attL, attR, attP, lox, FRT, etc.

***Example 21: Mutational Analysis of the Bacteriophage Lambda attL and attR Sites: Determinants of att Site Specificity in Site-specific Recombination***

To investigate the determinants of att site specificity, the bacteriophage lambda attL and attR sites were systematically mutagenized. As noted herein, the determinants of specificity have previously been localized to the 7 bp overlap region (TTTATAC, which is defined by the cut sites for the integrase protein and is the region where strand exchange takes place) within the 15 bp core region (GCTTTTATATACTAA) which is identical in all four lambda att sites, attB, attP, attL and attR. This core region, however, has not heretofore been systematically

mutagenized and examined to define precisely which mutations produce unique changes in *att* site specificity.

Therefore, to examine the effect of *att* sequence on site specificity, mutant *attL* and *attR* sites were generated by PCR and tested in an *in vitro* site-specific recombination assay. In this way all possible single base pair changes within the 7 bp overlap region of the core *att* site were generated as well as five additional changes outside the 7 bp overlap but within the 15 bp core *att* site. Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates.

### Methods

To examine both the efficiency and specificity of recombination of mutant *attL* and *attR* sites, a simple *in vitro* site-specific recombination assay was developed. Since the core regions of *attL* and *attR* lie near the ends of these sites, it was possible to incorporate the desired nucleotide base changes within PCR primers and generate a series of PCR products containing mutant *attL* and *attR* sites. PCR products containing *attL* and *attR* sites were used as substrates in an *in vitro* reaction with GATEWAY™ LR Clonase™ Enzyme Mix (Life Technologies, Inc.; Rockville, MD). Recombination between a 1.5 kb *attL* PCR product and a 1.0 kb *attR* PCR product resulted in a 2.5 kb recombinant molecule that was monitored using agarose gel electrophoresis and ethidium bromide staining.

Plasmid templates pEZC1301 (Figure 84) and pEZC1313 (Figure 85), each containing a single wild type *attL* or *attR* site, respectively, were used for the generation of recombination substrates. The following list shows primers that were used in PCR reactions to generate the *attL* PCR products that were used as substrates in L x R Clonase reactions (capital letters represent changes from the wild-type sequence, and the underline represents the 7 bp overlap region within the 15 bp core *att* site; a similar set of PCR primers was used to prepare the *attR* PCR products containing matching mutations):

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GATEWAY™ sites (note: attL2 sequence in GATEWAY™ plasmids begins "accga" while the attL2 site in this example begins "agcct" to reflect wild-type attL outside the core region.):

5

attL1: gggg agcct gcttttttGtacAaa gttggcatta taaaaa-  
agca ttgc

10

attL2: gggg agcct gcttttCttGtacAaa gttggcatta taaaaa-  
agca ttgc

Wild-type:

15

attL0: gggg agcct gctttttttataactaa gttggcatta taaaaa-  
agca ttgc

Single base changes from wild-type:

attLT1A: gggg agcct gcttttAttataactaa gttggcatta taaaaa-  
agca ttgc

20

attLT1C: gggg agcct gcttttCttataactaa gttggcatta taaaaa-  
agca ttgc

attLT1G: gggg agcct gcttttGttataactaa gttggcatta taaaaa-  
agca ttgc

25

attLT2A: gggg agcct gcttttAtataactaa gttggcatta taaaaa-  
agca ttgc

attLT2C: gggg agcct gcttttCtataactaa gttggcatta taaaaa-  
agca ttgc

30

attLT2G: gggg agcct gcttttGtataactaa gttggcatta taaaaa-  
aagca ttgc

35

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attLT3A: gggg agcct gcttttttAatactaa gttggcatta taaaa-  
aagca ttgc

5 attLT3C: gggg agcct gcttttttCatactaa gttggcatta taaaa-  
aagca ttgc

10 attLT3G: gggg agcct gcttttttGatactaa gttggcatta taaaa-  
aagca ttgc

15 attLA4C: gggg agcct gcttttttCtactaa gttggcatta taaaa-  
aagca ttgc

20 attLA4G: gggg agcct gcttttttGtactaa gttggcatta taaaa-  
aagca ttgc

25 attLA4T: gggg agcct gcttttttTtactaa gttggcatta taaaa-  
aagca ttgc

30 attLT5A: gggg agcct gcttttttAaactaa gttggcatta taaaa-  
aagca ttgc

35 attLT5C: gggg agcct gcttttttCaactaa gttggcatta taaaa-  
aagca ttgc

attLT5G: gggg agcct gcttttttGaactaa gttggcatta taaaa-  
aagca ttgc

attLA6C: gggg agcct gcttttttatCctaa gttggcatta taaaa-  
aagca ttgc

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attLA6G: gggg agcct gctttttttatGctaa gttggcatta taaaa-  
aagca ttgc

5 attLA6T: gggg agcct gctttttttatTctaa gttggcatta taaaa-  
aagca ttgc

10 attLC7A: gggg agcct gctttttttataAtaa gttggcatta taaaa-  
aagca ttgc

15 attLC7G: gggg agcct gctttttttataGtaa gttggcatta taaaa-  
aagca ttgc

attLC7T: gggg agcct gctttttttataTtaa gttggcatta taaaa-  
aagca ttgc

Single base changes outside of the 7 bp overlap:

20 attL8: gggg agcct Actttttttataactaa gttggcatta taaaa-  
aagca ttgc

25 attL9: gggg agcct gcCttttttataactaa gttggcatta taaaaa-  
agca ttgc

attL10: gggg agcct gcttCttttataactaa gttggcatta taaaaa-  
agca ttgc

30 attL14: gggg agcct gctttttttatacCaa gttggcatta taaaaa-  
agca ttgc

35 attL15: gggg agcct gctttttttataactaG gttggcatta taaaaa-  
agca ttgc



Note: additional vectors wherein the first nine bases are gggg agcca (*i.e.*, substituting an adenine for the thymine in the position immediately preceding the 15-bp core region), which may or may not contain the single base pair substitutions (or deletions) outlined above, can also be used in these experiments.

Recombination reactions of *attL*- and *attR*-containing PCR products was performed as follows:

8 µl of H<sub>2</sub>O

2 µl of *attL* PCR product (100 ng)

2 µl of *attR* PCR product (100 ng)

4 µl of 5x buffer

4 µl of GATEWAY™ LR Clonase™ Enzyme Mix

20 µl total volume

Clonase reactions were incubated at 25°C for 2 hours.

2 µl of 10X Clonase stop solution (proteinase K, 2 mg/ml) were added to stop the reaction.

10 µl were run on a 1 % agarose gel.

### Results

Each *attL* PCR substrate was tested in the *in vitro* recombination assay with each of the *attR* PCR substrates. Changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination. These mutant *att* sites each recombined as well as the wild-type, but only with their cognate partner mutant; they did not recombine detectably with any other *att* site mutant. In contrast, changes in the last four positions (TTTATAC) only partially altered specificity; these mutants recombined with their cognate mutant as well as wild-type *att* sites and recombined partially with all other mutant *att* sites except for those having mutations in the first three positions of the 7 bp

overlap. Changes outside of the 7 bp overlap were found not to affect specificity of recombination, but some did influence the efficiency of recombination.

Based on these results, the following rules for *att* site specificity were determined:

- Only changes within the 7 bp overlap affect specificity.
- Changes within the first 3 positions strongly affect specificity.
- Changes within the last 4 positions weakly affect specificity.

Mutations that affected the overall efficiency of the recombination reaction were also assessed by this method. In these experiments, a slightly increased (less than 2-fold) recombination efficiency with *att*LT1A and *att*LC7T substrates was observed when these substrates were reacted with their cognate *att*R partners. Also observed were mutations that decreased recombination efficiency (approximately 2-3 fold), including *att*LA6G, *att*L14 and *att*L15. These mutations presumably reflect changes that affect Int protein binding at the core *att* site.

The results of these experiments demonstrate that changes within the first three positions of the 7 bp overlap (TTTATAC) strongly altered the specificity of recombination (*i.e.*, *att* sequences with one or more mutations in the first three thymidines would only recombine with their cognate partners and would not cross-react with any other *att* site mutation). In contrast, mutations in the last four positions (TTTATAC) only partially altered specificity (*i.e.*, *att* sequences with one or more mutations in the last four base positions would cross-react partially with the wild-type *att* site and all other mutant *att* sites, except for those having mutations in one or more of the first three positions of the 7 bp overlap). Mutations outside of the 7 bp overlap were not found to affect specificity of recombination, but some were found to influence (*i.e.*, to cause a decrease in) the efficiency of recombination.

***Example 22: Discovery of Att Site Mutations That Increase the Cloning Efficiency of GATEWAY™ Cloning Reactions***

In experiments designed to understand the determinants of *att* site specificity, point mutations in the core region of *att*L were made. Nucleic acid molecules containing these mutated *att*L sequences were then reacted in an LR

reaction with nucleic acid molecules containing the cognate *attR* site (*i.e.*, an *attR* site containing a mutation corresponding to that in the *attL* site), and recombinational efficiency was determined as described above. Several mutations located in the core region of the *att* site were noted that either slightly increased (less than 2-fold) or decreased (between 2-4-fold) the efficiency of the recombination reaction (Table 3).

Table 3. *Effects of attL mutations on Recombination Reactions.*

<u>Site</u>	<u>Sequence</u>	<u>Effect on Recombination</u>
attL0	agcctgcttttttataactaagttggcatta	
attL5	agcctgcttttAttataactaagttggcatta	slightly increased
attL6	agcctgctttttttataTtaagttggcatta	slightly increased
attL13	agcctgctttttttatGctaagttggcatta	decreased
attL14	agcctgctttttttatacCaagttggcatta	decreased
attL15	agcctgctttttttataactaGgttggcatta	decreased
consensus	CAACTTnnTnnnAnnAAGTTG	

It was also noted that these mutations presumably reflected changes that either increased or decreased, respectively, the relative affinity of the integrase protein for binding the core *att* site. A consensus sequence for an integrase core-binding site (CAACTTNNT) has been inferred in the literature but not directly tested (see, *e.g.*, Ross and Landy, *Cell* 33:261-272 (1983)). This consensus core integrase-binding sequence was established by comparing the sequences of each of the four core *att* sites found in *attP* and *attB* as well as the sequences of five non-*att* sites that resemble the core sequence and to which integrase has been shown to bind *in vitro*. These experiments suggest that many more *att* site mutations might be identified which increase the binding of integrase to the core *att* site and thus increase the efficiency of GATEWAY™ cloning reactions.

**Example 23: Effects of Core Region Mutations on Recombination Efficiency**

To directly compare the cloning efficiency of mutations in the att site core region, single base changes were made in the attB2 site of an attB1-TET-attB2 PCR product. Nucleic acid molecules containing these mutated attB2 sequences were then reacted in a BP reaction with nucleic acid molecules containing non-cognate attP sites (*i.e.*, wildtype attP2), and recombinational efficiency was determined as described above. The cloning efficiency of these mutant attB2 containing PCR products compared to standard attB1-TET-attB2 PCR product are shown in Table 4.

**Table 4. Efficiency of Recombination With Mutated attB2 Sites.**

<u>Site</u>	<u>Sequence</u>	<u>Mutation</u>	<u>Cloning Efficiency</u>
attB0	tcaagttagtataaaaaagcaggct		
attB1	ggggacaagtttgtacaaaaagcaggct		
attB2	ggggaccactttgtacaagaaagctgggt		100%
attB2.1	ggggaAcactttgtacaagaaagctgggt	C→A	40%
attB2.2	ggggacAactttgtacaagaaagctgggt	C→A	131%
attB2.3	ggggaccCctttgtacaagaaagctgggt	A→C	4%
attB2.4	ggggaccaAttgtacaagaaagctgggt	C→A	11%
attB2.5	ggggaccacGttgtacaagaaagctgggt	T→G	4%
attB2.6	ggggaccactGtgtacaagaaagctgggt	T→G	6%
attB2.7	ggggaccacttGgtacaagaaagctgggt	T→G	1%
attB2.8	ggggaccactttTtacaagaaagctgggt	G→T	0.5%

As noted above, a single base change in the attB2.2 site increased the cloning efficiency of the attB1-TET-attB2.2 PCR product to 131% compared to the attB1-TET-attB2 PCR product. Interestingly, this mutation changes the integrase core binding site of attB2 to a sequence that matches more closely the proposed consensus sequence.

Additional experiments were performed to directly compare the cloning efficiency of an attB1-TET-attB2 PCR product with a PCR product that contained attB sites containing the proposed consensus sequence (see Example 22) of an integrase core binding site. The following attB sites were used to amplify attB-TET PCR products:

attB1      ggggacaagtttgtacaaaaaagcaggct  
attB1.6    ggggacaaCtttgtacaaaaaagTTggct  
attB2      ggggaccactttgtacaagaaagctgggt  
attB2.10   ggggacAactttgtacaagaaagTtgggt

BP reactions were carried out between 300 ng (100 fmoles) of pDONR201 (Figure 49A) with 80 ng (80 fmoles) of attB-TET PCR product in a 20 µl volume with incubation for 1.5 hrs at 25°C, creating pENTR201-TET Entry clones. A comparison of the cloning efficiencies of the above-noted attB sites in BP reactions is shown in Table 5.

Table 5. Cloning efficiency of BP Reactions.

PCR product	CFU/ml	Fold Increase
B1-tet-B2	7,500	
B1.6-tet-B2	12,000	1.6 x
B1-tet-B2.10	20,900	2.8 x
B1.6-tet-B2.10	30,100	4.0 x

These results demonstrate that attB PCR products containing sequences that perfectly match the proposed consensus sequence for integrase core binding sites can produce Entry clones with four-fold higher efficiency than standard Gateway attB1 and attB2 PCR products.

The entry clones produced above were then transferred to pDEST20 (Figure 40A) via LR reactions (300 ng (64 fmoles) pDEST20 mixed with 50 ng (77 fmoles) of the respective pENTR201-TET Entry clone in 20 µl volume; incubated for 1 hr incubation at 25°C). The efficiencies of cloning for these reactions are compared in Table 6.

Table 6. Cloning Efficiency of LR Reactions.

pENTR201-TET x pDEST20	CFU/ml	Fold Increase
L1-tet-L2	5,800	
L1.6-tet-L2	8,000	1.4
L1-tet-L2.10	10,000	1.7
L1.6-tet-L2.10	9,300	1.6

These results demonstrate that the mutations introduced into attB1.6 and attB2.10 that transfer with the gene into entry clones slightly increase the efficiency of LR reactions. Thus, the present invention encompasses not only mutations in *attB* sites that increase recombination efficiency, but also to the corresponding mutations that result in the *attL* sites created by the BP reaction.

To examine the increased cloning efficiency of the attB1.6-TET-attB2.10 PCR product over a range of PCR product amounts, experiments analogous to those described above were performed in which the amount of attB PCR product was titrated into the reaction mixture. The results are shown in Table 7.

Table 7. Titration of attB PCR products.

Amount of attB PCR product (ng)	PCR product	CFU/ml	Fold Increase
20	attB1-TET-attB2	3,500	6.1
	attB1.6-TET-attB2.10	21,500	
50	attB1-TET-attB2	9,800	5.0
	attB1.6-TET-attB2.10	49,000	
100	attB1-TET-attB2	18,800	2.8
	attB1.6-TET-attB2.10	53,000	
200	attB1-TET-attB2	19,000	2.5
	attB1.6-TET-attB2.10	48,000	

These results demonstrate that as much as a six-fold increase in cloning efficiency is achieved with the attB1.6-TET-attB2.10 PCR product as compared to the standard attB1-TET-attB2 PCR product at the 20 ng amount.



**Example 24: Determination of attB Sequence Requirements for Optimum Recombination Efficiency**

To examine the sequence requirements for attB and to determine which attB sites would clone with the highest efficiency from populations of degenerate attB sites, a series of experiments was performed. Degenerate PCR primers were designed which contained five bases of degeneracy in the B-arm of the attB site. These degenerate sequences would thus transfer with the gene into Entry clone in BP reactions and subsequently be transferred with the gene into expression clones in LR reactions. The populations of degenerate attB and attL sites could thus be cycled from attB to attL back and forth for any number of cycles. By altering the reaction conditions at each transfer step (for example by decreasing the reaction time and/or decreasing the concentration of DNA) the reaction can be made increasingly more stringent at each cycle and thus enrich for populations of attB and attL sites that react more efficiently.

The following degenerate PCR primers were used to amplify a 500 bp fragment from pUC18 which contained the lacZ alpha fragment (only the attB portion of each primer is shown):

attB1	GGGG	ACAAGTTT	<u>GTACAAA</u>	AAAGC	AGGCT
attB1n16-20	GGGG	ACAAGTTT	<u>GTACAAA</u>	nnnnn	AGGCT
attB1n21-25	GGGG	ACAAGTTT	<u>GTACAAA</u>	AAAGC	nnnnn
attB2	GGGG	ACCACTTT	<u>GTACAAG</u>	AAAGC	TGGGT
attB2n16-20	GGGG	ACCACTTT	<u>GTACAAG</u>	nnnnn	TGGGT
attB2n21-25	GGGG	ACCACTTT	<u>GTACAAG</u>	AAAGC	nnnnn

The starting population size of degenerate att sites is  $4^5$  or 1024 molecules. Four different populations were transferred through two BP reactions and two LR reactions. Following transformation of each reaction, the population of transformants was amplified by growth in liquid media containing the appropriate selection antibiotic. DNA was prepared from the population of clones by alkaline

lysis miniprep and used in the next reaction. The results of the BP and LR cloning reactions are shown below.

BP-1, overnight reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	78,500	100 %
attB1n16-20-LacZa-attB2	1,140	1.5 %
attB1n21-25-LacZa-attB2	11,100	14 %
attB1-LacZa-attB2n16-20	710	0.9 %
attB1-LacZa-attB2n21-25	16,600	21 %

LR-1, pENTR201-LacZa x pDEST20/EcoRI, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	20,000	100 %
attL1n16-20-LacZa-attL2	2,125	11 %
attL1n21-25-LacZa-attL2	2,920	15 %
attL1-LacZa-attL2n16-20	3,190	16 %
attL1-LacZa-attL2n21-25	1,405	7 %

BP-2, pEXP20-LacZa/ScaI x pDONR 201, 1hr reactions

	cfu/ml	percent of control
attB1-LacZa-attB2	48,600	100 %
attB1n16-20-LacZa-attB2	22,800	47 %
attB1n21-25-LacZa-attB2	31,500	65 %
attB1-LacZa-attB2n16-20	42,400	87 %
attB1-LacZa-attB2n21-25	34,500	71 %

LR-2, pENTR201-LacZa x pDEST6/NcoI, 1hr reactions

	cfu/ml	percent of control
attL1-LacZa-attL2	23,000	100 %
attL1n16-20-LacZa-attL2	49,000	213 %
attL1n21-25-LacZa-attL2	18,000	80 %
attL1-LacZa-attL2n16-20	37,000	160 %
attL1-LacZa-attL2n21-25	57,000	250 %

These results demonstrate that at each successive transfer, the cloning efficiency of the entire population of att sites increases, and that there is a great deal of flexibility in the definition of an attB site. Specific clones may be isolated from the above reactions, tested individually for recombination efficiency, and

sequenced. Such new specificities may then be compared to known examples to guide the design of new sequences with new recombination specificities. In addition, based on the enrichment and screening protocols described herein, one of ordinary skill can easily identify and use sequences in other recombination sites, e.g., other *att* sites, *lox*, FRT, etc., that result in increased specificity in the recombination reactions using nucleic acid molecules containing such sequences.

***Example 25: Design of att Site PCR Adapter-Primers***

Additional studies were performed to design gene-specific primers with 12bp of attB1 and attB2 at their 5'-ends. The optimal primer design for *att*-containing primers is the same as for any PCR primers: the gene-specific portion of the primers should ideally have a  $T_m$  of  $> 50^\circ\text{C}$  at 50 mM salt (calculation of  $T_m$  is based on the formula  $59.9 + 41(\%GC) - 675/n$ ).

**Primers:**

12bp attB1: AA AAA GCA GGC TNN - forward gene-specific primer

12bp attB2: A GAA AGC TGG GTN - reverse gene-specific primer

attB1 adapter primer: GGGGACAAGTTTGTACAAAAAAGCAGGCT

attB2 adapter primer: GGGGACCACTTTGTACAAGAAAGCTGGGT

**Protocol:**

(1) Mix 200 ng of cDNA library or 1 ng of plasmid clone DNA (alternatively, genomic DNA or RNA could be used) with 10 pmoles of gene specific primers in a 50  $\mu\text{l}$  PCR reaction, using one or more polypeptides having DNA polymerase activity such as those described herein. (The addition of greater than 10 pmoles of gene-specific primers can decrease the yield of attB PCR product. In addition, if RNA is used, a standard reverse transcriptase-PCR (RT-

PCR) protocol should be followed; *see, e.g.,* Gerard, G.F., *et al.*, *FOCUS* 11:60 (1989); Myers, T.W., and Gelfand, D.H., *Biochem.* 30:7661 (1991); Freeman, W.N., *et al.*, *BioTechniques* 20:782 (1996); and U.S. Application No. 09/064,057, filed April 22, 1998, the disclosures of all of which are incorporated herein by reference.)

1<sup>st</sup> PCR profile:

(a) 95°C for 3 minutes

(b) 10 cycles of:

(i) 94°C for 15 seconds

(ii) 50°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 68°C for 5 minutes

(d) 10°C hold

\*The optimal annealing temperature is determined by the calculated T<sub>m</sub> of the gene-specific part of the primer.

(2) Transfer 10 µl to a 40 µl PCR reaction mix containing 35 pmoles each of the attB1 and attB2 adapter primers.

2<sup>nd</sup> PCR profile:

(a) 95°C for 1 minute

(b) 5 cycles of:

(i) 94°C for 15 seconds

(ii) 45°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(c) 15-20 cycles\*\* of:

(i) 94°C for 15 seconds

(ii) 55°C\* for 30 seconds

(iii) 68°C for 1 minute/kb of target amplicon

(d) 68°C for 5 minutes

(e) 10°C hold

5      \*The optimal annealing temperature is determined by the calculated  $T_m$  of the gene-specific part of the primer.

    \*\*15 cycles is sufficient for low complexity targets.

Notes:

- 10      1. It is useful to perform a no-adapter primer control to assess the yield of attB PCR product produced.
2. Linearized template usually results in slightly greater yield of PCR product.

15      ***Example 26: One-Tube Recombinational Cloning Using the GATEWAY™ Cloning System***

20      To provide for easier and more rapid cloning using the GATEWAY™ cloning system, we have designed a protocol whereby the BP and LR reactions may be performed in a single tube (a "one-tube" protocol). The following is an example of such a one-tube protocol; in this example, an aliquot of the BP reaction is taken before adding the LR components, but the BP and LR reactions may be performed in a one-tube protocol without first taking the BP aliquot:

25

<u>Reaction Component</u>	<u>Volume</u>
attB DNA (100-200 ng/25 µl reaction)	1-12.5 µl
attP DNA (pDONR201) 150 ng/µl	2.5 µl
5X BP Reaction Buffer	5.0 µl
Tris-EDTA	(to 20 µl)
<u>BP Clonase</u>	<u>5.0 µl</u>
Total vol.	25 µl

30

After the above components were mixed in a single tube, the reaction mixtures were incubated for 4 hours at 25°C. A 5 µl aliquot of reaction mixture was removed, and 0.5 µl of 10X stop solution was added to this reaction mixture and incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the BP reaction per 100 µl of cells; this transformation yielded colonies of Entry Clones for isolation of individual Entry Clones and for quantitation of the BP Reaction efficiency.

To the remaining 20 µl of BP reaction mixture, the following components of the LR reaction were added:

<u>Reaction Component</u>	<u>Final Concentration</u>	<u>Volume Added</u>
NaCl	0.75 M	1 µl
Destination Vector	150 ng/ul	3 µl
LR Clonase		6 µl
Total vol.		30 µl

After the above components were mixed in a single tube, the reaction mixtures were incubated for 2 hours at 25°C. 3 µl of 10X stop solution was added, and the mixture was incubated for 10 minutes at 37°C. Competent cells were then transformed with 1-2 µl of the reaction mixture per 100 µl of cells

Notes:

1. If desired, the Destination Vector can be added to the initial BP reaction.
2. The reactions can be scaled down by 2x, if desired.
3. Shorter incubation times for the BP and/or LR reactions can be used (scaled to the desired cloning efficiencies of the reaction), but a lower number of colonies will typically result.
4. To increase the number of colonies obtained by several fold, incubate the BP reaction for 6-20 hours and increase the LR reaction to 3 hours. Electroporation also works well with 1-2 ul of the PK-treated reaction mixture.



5. PCR products greater than about 5 kb may show significantly lower cloning efficiency in the BP reaction. In this case, we recommend using a one-tube reaction with longer incubation times (*e.g.*, 6-18 hours) for both the BP and LR steps.

***Example 27: Relaxation of Destination Vectors During the LR Reaction***

To further optimize the LR Reaction, the composition of the LR Reaction buffer was modified from that described above and this modified buffer was used in a protocol to examine the impact of enzymatic relaxation of Destination Vectors during the LR Reaction.

LR Reactions were set up as usual (*see, e.g.*, Example 6), except that 5X BP Reaction Buffer (*see* Example 5) was used for the LR Reaction. To accomplish Destination Vector relaxation during the LR Reaction, Topoisomerase I (Life Technologies, Inc., Rockville, MD; Catalogue No. 38042-016) was added to the reaction mixture at a final concentration of ~15U per  $\mu\text{g}$  of total DNA in the reaction (for example, for reaction mixtures with a total of 400ng DNA in the 20  $\mu\text{l}$  LR Reaction, ~6units of Topoisomerase I was added).

Reaction mixtures were set up as follows:

<u>Reaction Component</u>	<u>Volume</u>
ddH <sub>2</sub> O	6.5 $\mu\text{l}$
4X BP Reaction Buffer	5 $\mu\text{l}$
100ng single chain/linear pENTR CAT, 50 ng/ $\mu\text{l}$	2 $\mu\text{l}$
300ng single chain/linear pDEST6, 150ng/ $\mu\text{l}$	2 $\mu\text{l}$
Topoisomerase I, 15 U/ml	0.5 $\mu\text{l}$
LR Clonase	4 $\mu\text{l}$

Reaction mixtures were incubated at 25°C for 1hour, and 2  $\mu\text{l}$  of 2  $\mu\text{g}/\mu\text{l}$  Proteinase K was then added and mixtures incubated for 10 minutes at 37°C to stop the LR Reaction. Competent cells were then transformed as described in the preceding examples. The results of these studies demonstrated that relaxation of

substrates in the LR reaction using Topoisomerase I resulted in a 2- to 10-fold increase in colony output compared to those LR reactions performed without including Topoisomerase I.

5           Having now fully described the present invention in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or  
10 any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

          All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent  
15 as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.

167.1

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Escherichia coli DB3.1(pENTR-1A)	
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United States of America

Date of deposit  
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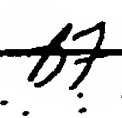
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Escherichia coli DB3.1(pENTR-3C)

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167.5

Applicant's or agent's file reference number	0942.468PC03	International application No. to PCT/US	00/05432
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INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
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(PCT Rule 13bis)

RE-17
ST

A. The indications made below relate to the microorganism referred to in the description on  
8.

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**B. IDENTIFICATION OF DEPOSIT**Further deposits are identified on an additional sheet ☒

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**C. ADDITIONAL INDICATIONS** (leave blank if not applicable)This information is continued on an additional sheet ☐

Escherichia coli DB3.1(pEZC15101)

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** (if the indications are not for all designated States)**E. SEPARATE FURNISHING OF INDICATIONS** (leave blank if not applicable)

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167.6

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(PCT Rule 13bis)

REC'D 17

WIPO

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
B. IDENTIFICATION OF DEPOSIT	
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Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
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C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
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Escherichia coli DB3.1(pEZC15102)	
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Accession Number  
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Escherichia coli DB3.1(pEZC15103)

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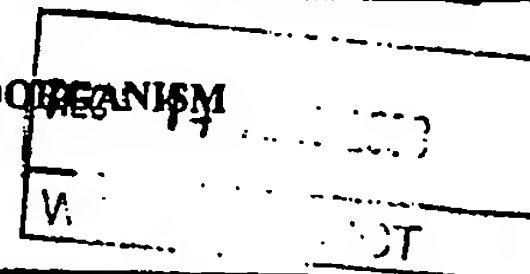
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167.8

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INDICATIONS RELATING TO DEPOSITED MICROORGANISM  
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(PCT Rule 13bis)



A. The indications made below relate to the microorganism referred to in the description on page 51, line 20-21.

B. IDENTIFICATION OF DEPOSIT

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United States of America

Date of deposit  
February 27, 1999

Accession Number  
NRRL B-30108

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

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Escherichia coli DB10B(pCMVSPORT6)

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

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## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group of nucleotide sequences consisting of an attB1 nucleotide sequence as set forth in Figure 9, an attB2 nucleotide sequence as set forth in Figure 9, an attP1 nucleotide sequence as set forth in Figure 9, an attP2 nucleotide sequence as set forth in Figure 9, an attL1 nucleotide sequence as set forth in Figure 9, an attL2 nucleotide sequence as set forth in Figure 9, an attR1 nucleotide sequence as set forth in Figure 9, an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, and a mutant, fragment, or derivative thereof.

2. An isolated nucleic acid molecule comprising an attB1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

3. An isolated nucleic acid molecule comprising an attB2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

4. An isolated nucleic acid molecule comprising an attP1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5. An isolated nucleic acid molecule comprising an attP2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

6. An isolated nucleic acid molecule comprising an attL1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

7. An isolated nucleic acid molecule comprising an attL2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5 8. An isolated nucleic acid molecule comprising an attR1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

10 9. An isolated nucleic acid molecule comprising an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

15 10. The isolated nucleic acid molecule of claim 1, further comprising one or more functional or structural nucleotide sequences selected from the group consisting of one or more multiple cloning sites, one or more localization signals, one or more transcription termination sites, one or more transcriptional regulatory sequences, one or more translational signals, one or more origins of replication, one or more fusion partner peptide-encoding nucleic acid molecules, one or more protease cleavage sites, and one or more 5' polynucleotide extensions.

20 11. The nucleic acid molecule of claim 10, wherein said transcriptional regulatory sequence is a promoter, an enhancer, or a repressor.

25 12. The nucleic acid molecule of claim 10, wherein said fusion partner peptide-encoding nucleic acid molecule encodes glutathione S-transferase (GST), hexahistidine (His<sub>6</sub>), or thioredoxin (Trx).

30 13. The nucleic acid molecule of claim 10, wherein said 5' polynucleotide extension consists of from one to five nucleotide bases.

14. The nucleic acid molecule of claim 13, wherein said 5' polynucleotide extension consists of four or five guanine nucleotide bases.



15. A primer nucleic acid molecule suitable for amplifying a target nucleotide sequence, comprising the isolated nucleic acid molecule of claim 1 or a portion thereof linked to a target-specific nucleotide sequence useful in amplifying said target nucleotide sequence.

16. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB1 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

17. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB2 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

18. The primer nucleic acid molecule of claim 15, further comprising a 5' terminal extension of four or five guanine bases.

19. A vector comprising the isolated nucleic acid molecule of claim 1.

20. The vector of claim 19, wherein said vector is an Expression Vector.

21. A host cell comprising the isolated nucleic acid molecule of claim 1 or the vector of claim 19.

22. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said

templates and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and

- 5 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.

10 23. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

- 15 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said templates and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
- 20 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.
- 25

30 24. A method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity

and one or more first primers comprising at least a portion of a recombination site and a template-specific sequence that is complementary to or capable of hybridizing to said template;

(b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one or both termini of said molecules;

(c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and

(d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one or both termini of said molecules.

25. A polypeptide encoded by the isolated nucleic acid molecule of any one of claims 1-10.

26. An isolated nucleic acid molecule comprising one or more *att* recombination sites comprising at least one mutation in its core region that increases the specificity of interaction between said recombination site and a second *att* recombination site.

27. The isolated nucleic acid molecule of claim 26, wherein said mutation is at least one substitution mutation of at least one nucleotide in the seven basepair overlap region of said core region of said recombination site.

28. The isolated nucleic acid molecule of claim 26, wherein said nucleic acid molecule comprises the sequence NNNATAC, wherein "N" refers to any nucleotide with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

29. An isolated nucleic acid molecule comprising one or more mutated *att* recombination sites comprising at least one mutation in its core region that enhances the efficiency of recombination between a first nucleic acid molecule comprising said mutated *att* recombination site and a second nucleic acid molecule comprising a second recombination site that interacts with said mutated *att* recombination site.

30. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site is a mutated *attL* site comprising a core region having the nucleotide sequence caactntntnnnannaagttg, wherein "n" represents any nucleotide.

31. The isolated nucleic acid molecule of claim 30, wherein said mutated *attL* recombination site comprises a core region having a nucleotide sequence selected from agcctgctttattataactaagttggcatta (*attL5*) and agcctgctttttatattaagttggcatta (*attL6*).

32. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site comprises a core region having a nucleotide sequence selected from the group consisting of ggggacaactttgtacaaaaagttggct (*attB1.6*), ggggacaactttgtacaagaaagctgggt (*attB2.2*), and ggggacaactttgtacaagaaagttgggt (*attB2.10*).

33. A vector selected from the group consisting of pENTR1A, pENTR2B, pENTR3C, pENTR4, pENTR5, pENTR6, pENTR7, pENTR8, pENTR9, pENTR10, pENTR11, pDEST1, pDEST2, pDEST3, pDEST4,

pDEST5, pDEST6, pDEST7, pDEST8, pDEST9, pDEST10, pDEST11, pDEST12.2 (also known as pDEST12), pDEST13, pDEST14, pDEST15, pDEST16, pDEST17, pDEST18, pDEST19, pDEST20, pDEST21, pDEST22, pDEST23, pDEST24, pDEST25, pDEST26, pDEST27, pDEST28, pDEST29, pDEST30, pDEST31, pDEST32, pDEST33, pDEST34, pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector), pDONR202, pDONR203 (also known as pEZ15812), pDONR204, pDONR205, pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector), pDONR207, pMAB58, pMAB62, pMAB85 and pMAB86.

34. A host cell comprising the vector of claim 33.

35. A polypeptide encoded by the vector of claim 33.

36. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the isolated nucleic acid molecule of any one of claims 1-10, 26 and 29.

37. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the primer of claim 15 or claim 18.

38. A kit for use in cloning a nucleic acid molecule, said kit comprising the vector of claim 19 or claim 33.

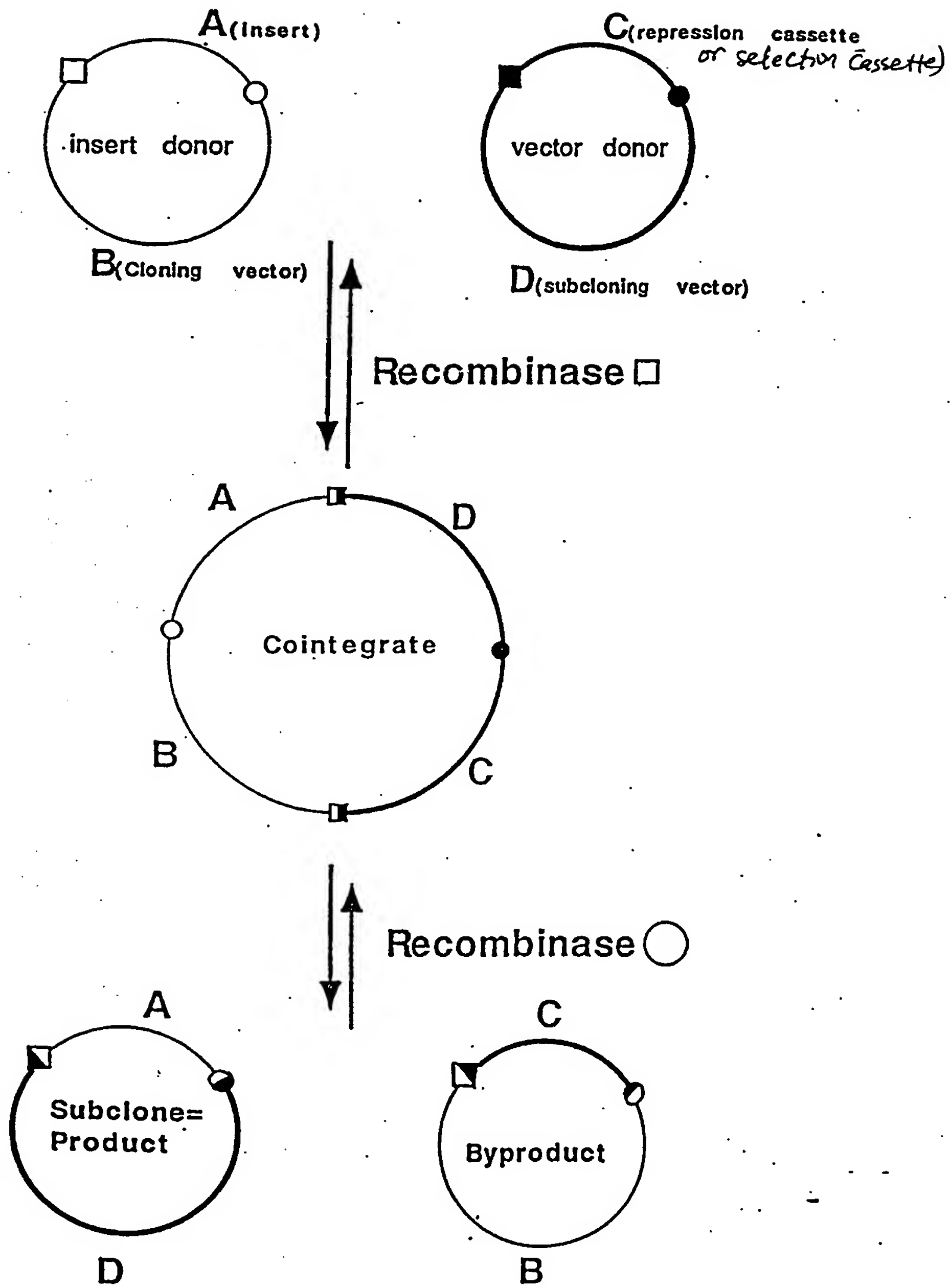


Figure 1



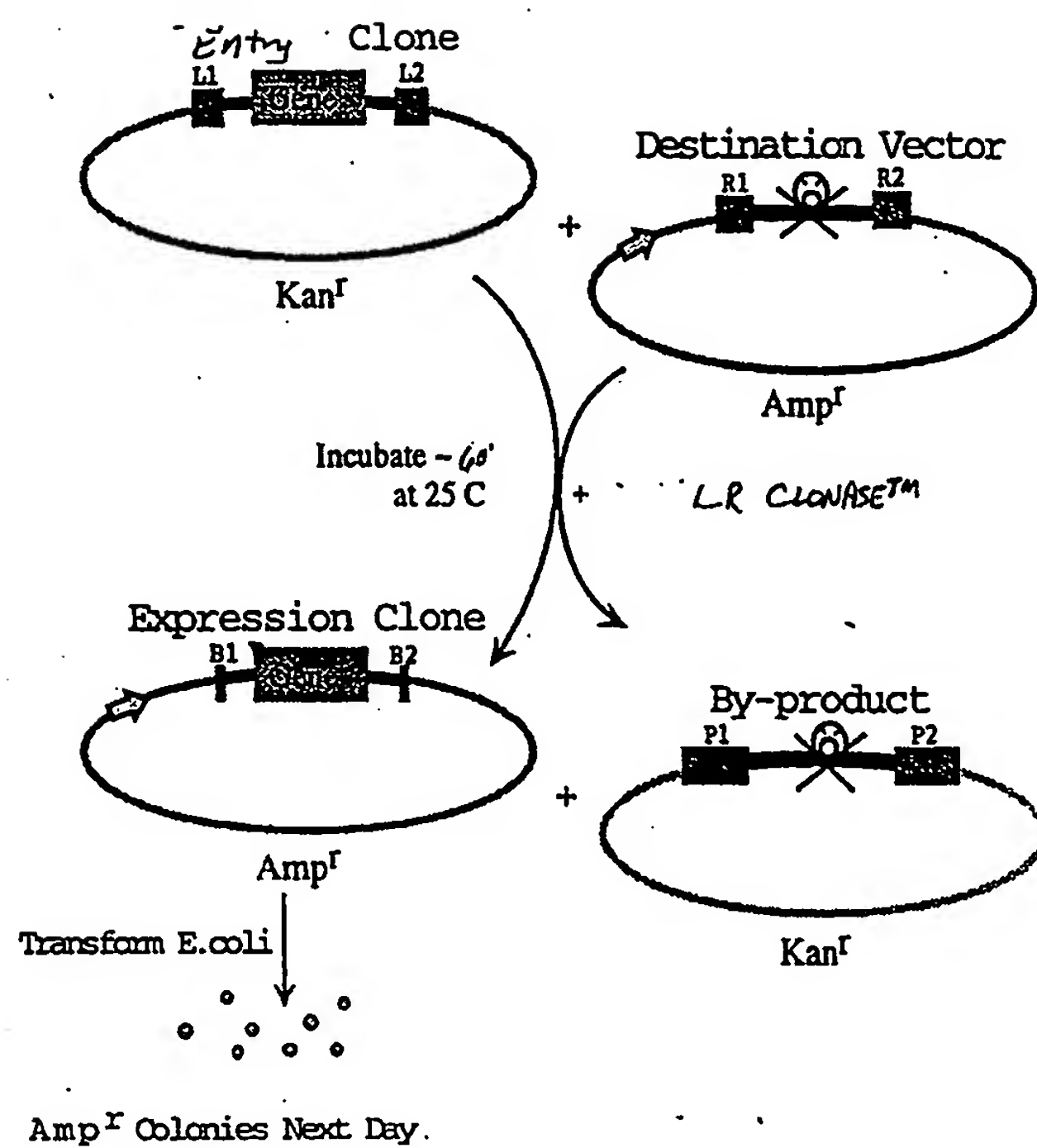


FIGURE 2

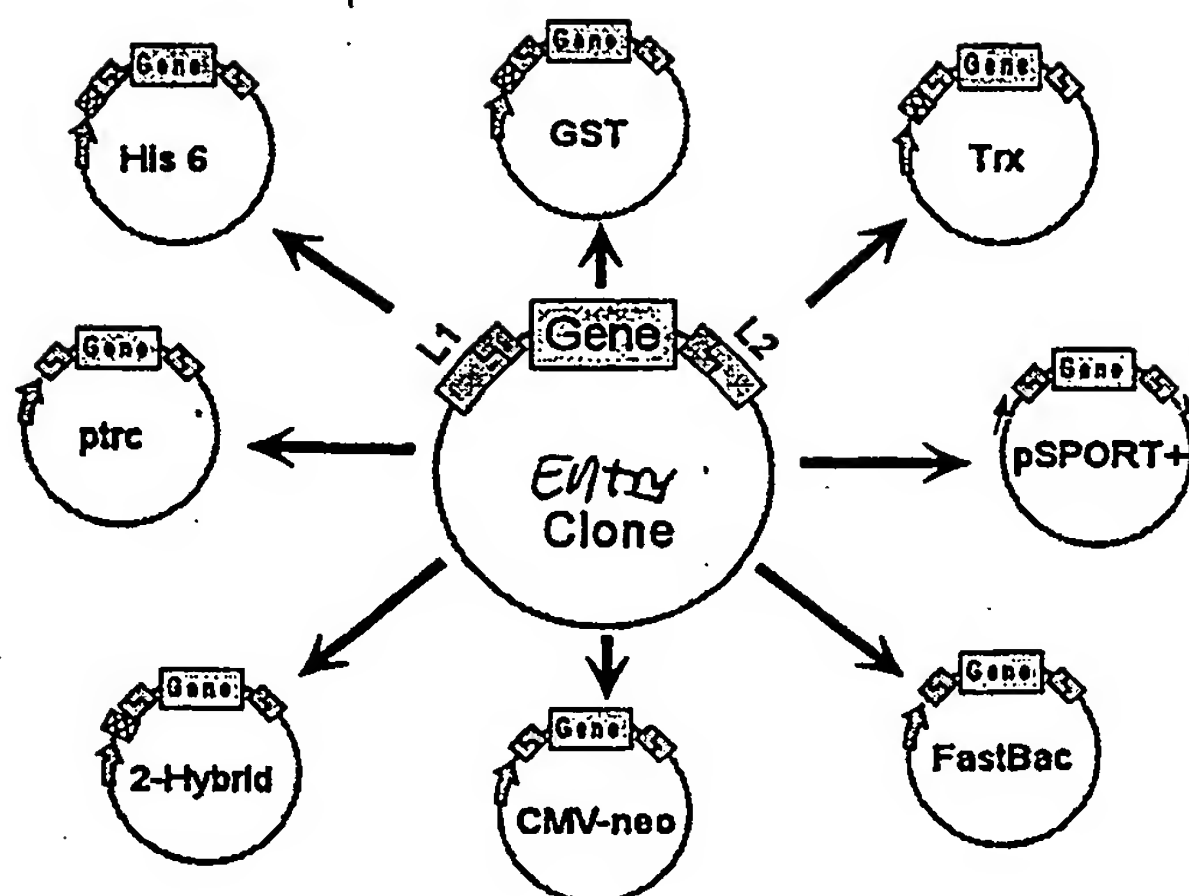


FIGURE 3

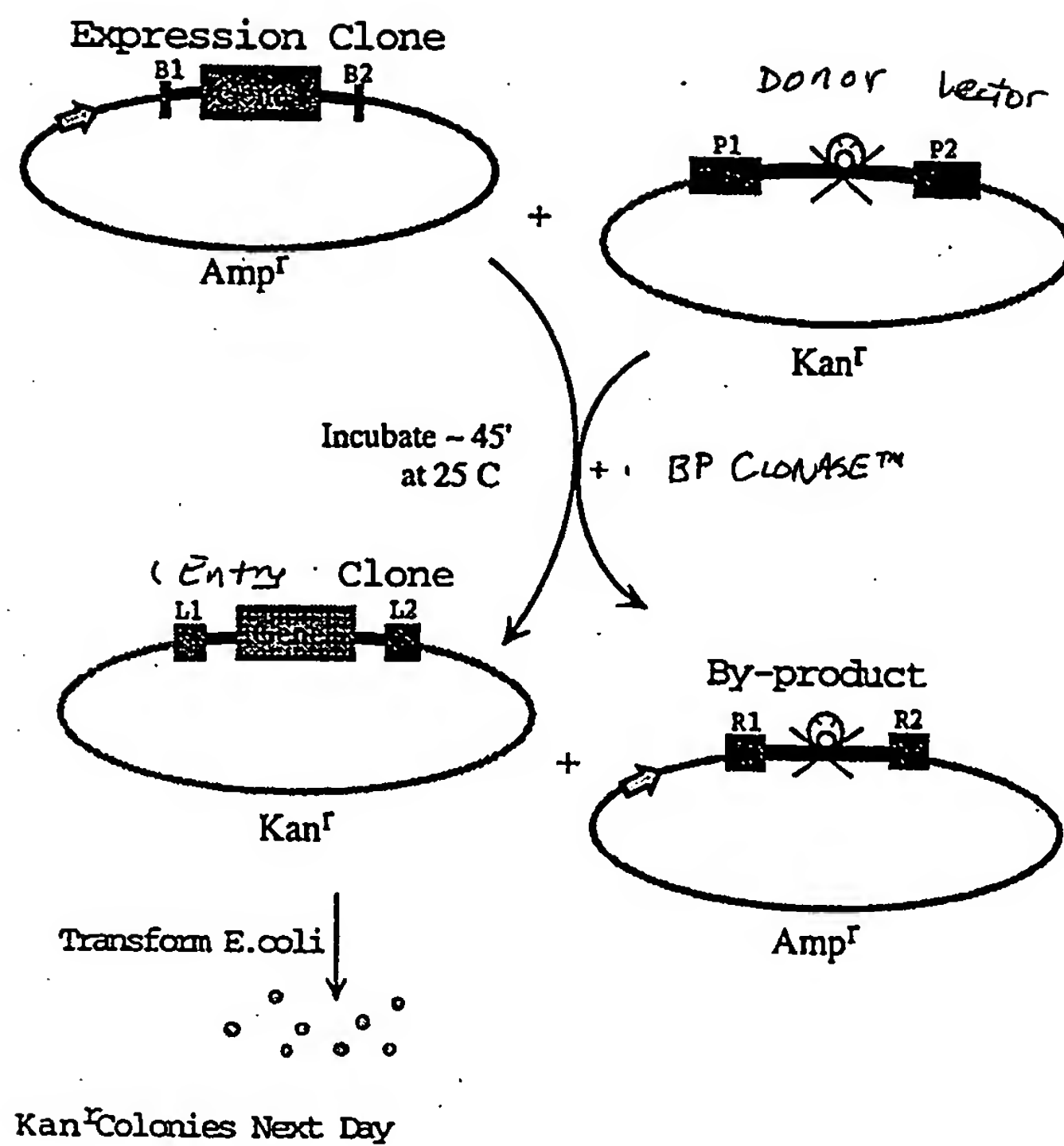


FIGURE 4

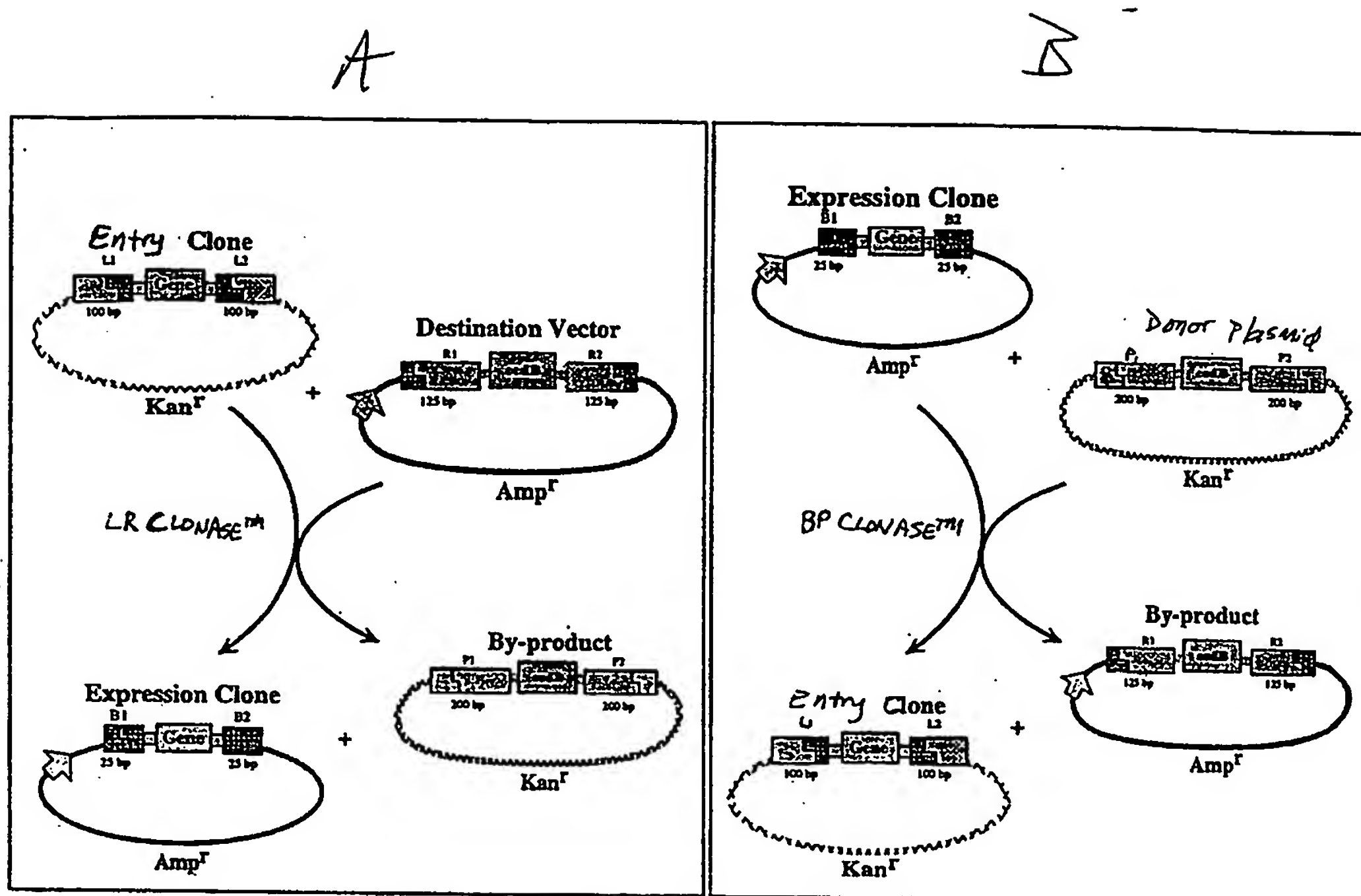


FIGURE 5

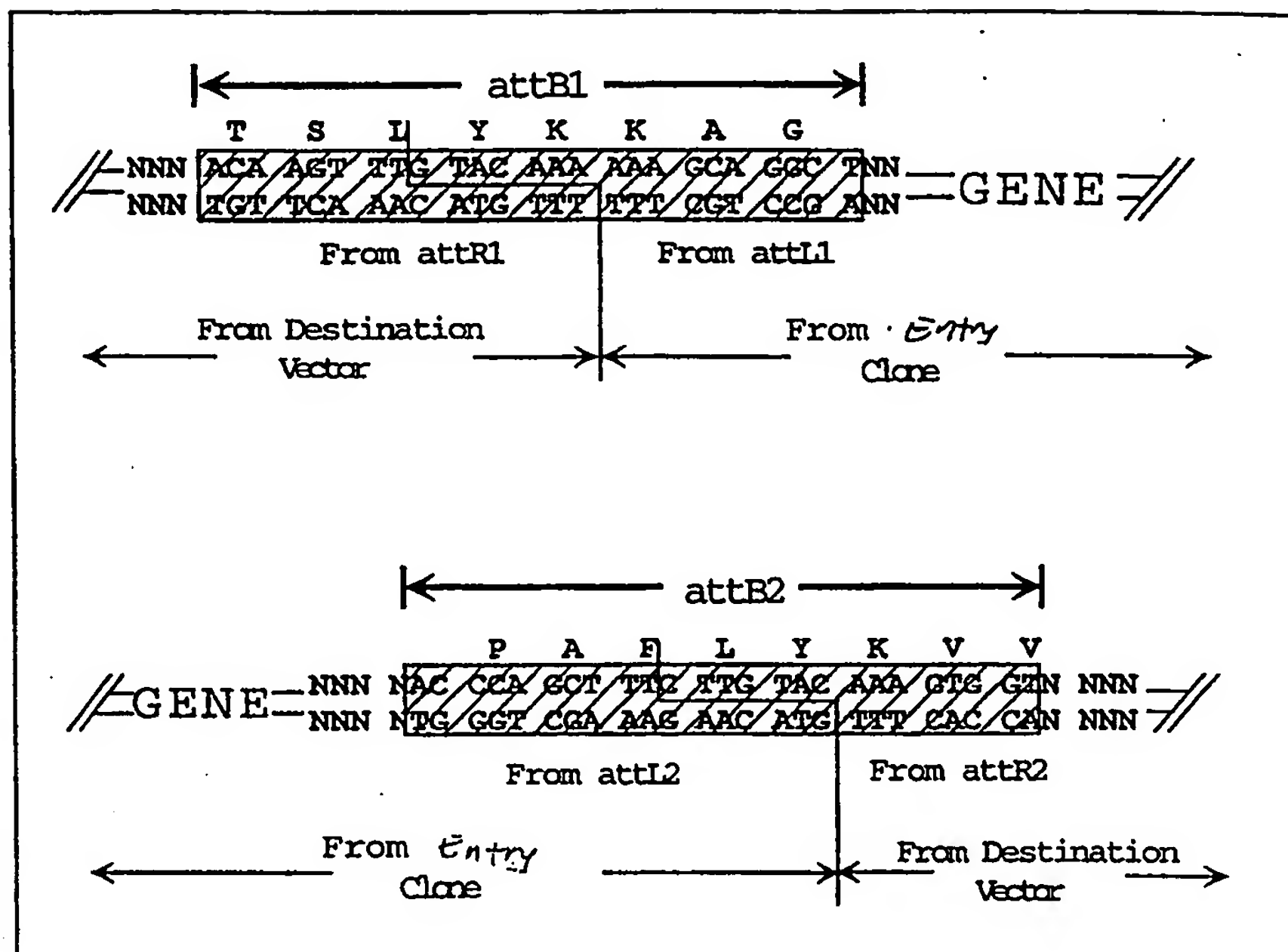


FIGURE 6

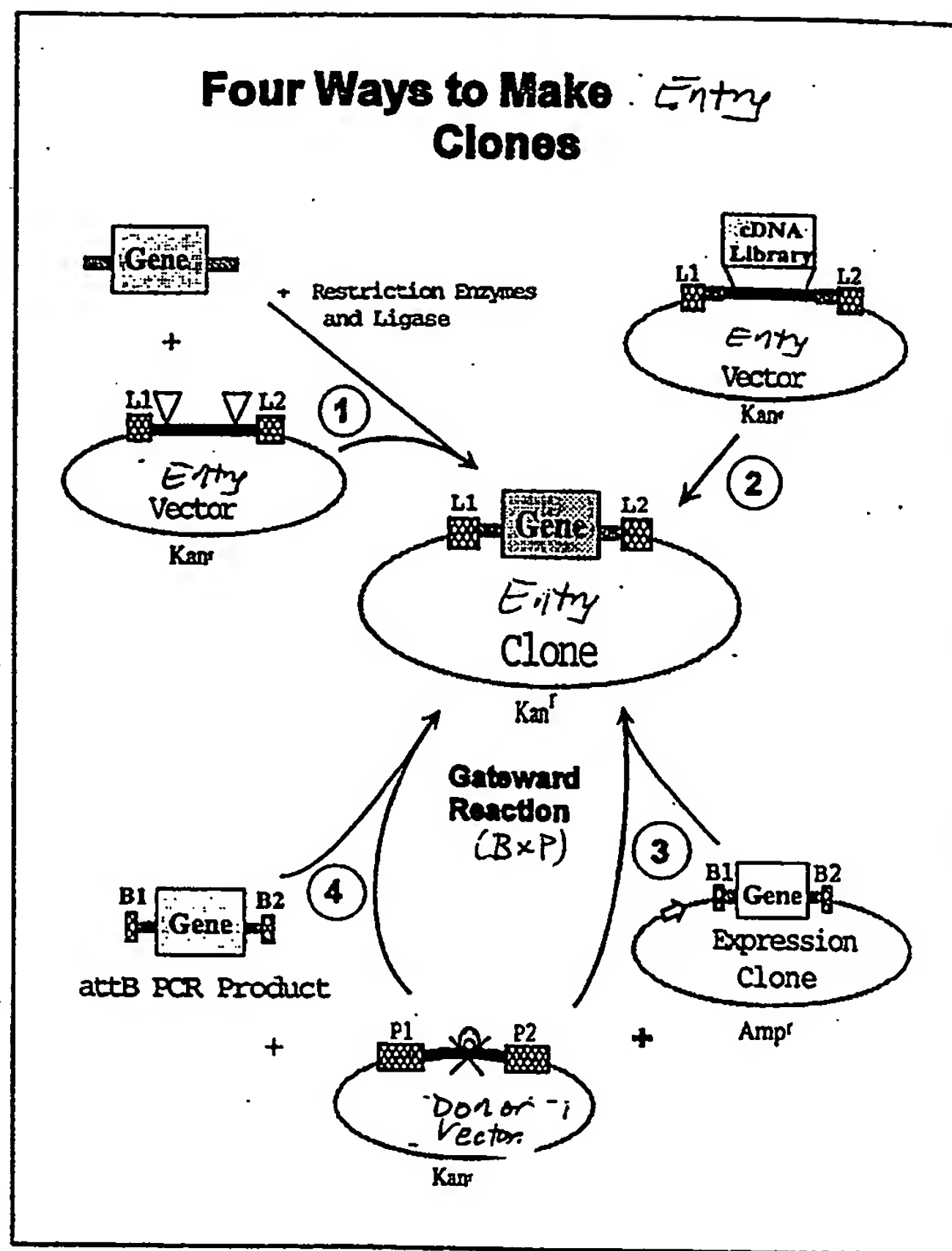


FIGURE 7



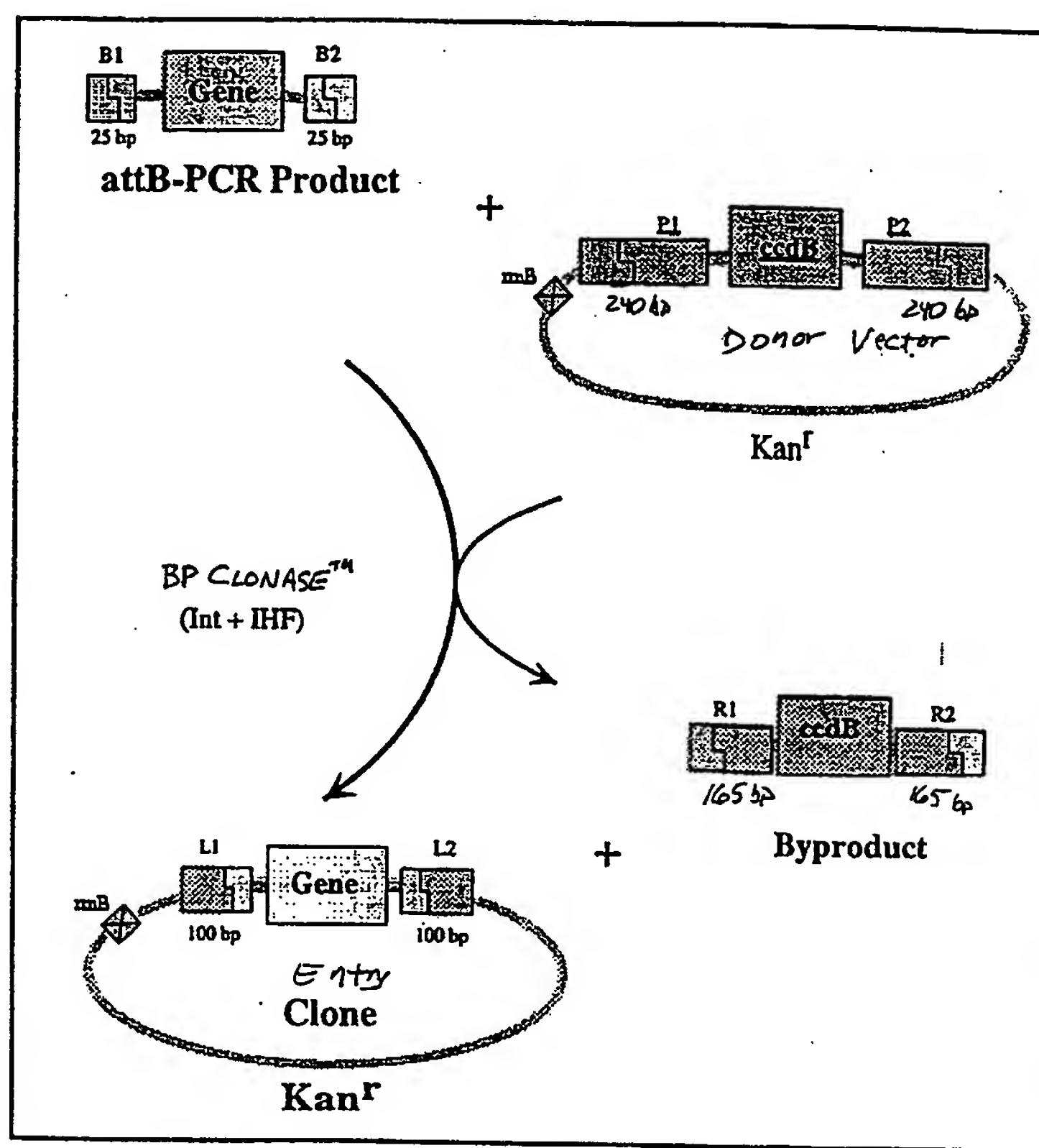


FIGURE 8

**Recombination Site Nucleotide Sequences**

attB1: 5'-ACAAGTTTGTACAAAAAAGCAGGCT-3'

attB2: 5'-ACCCAGCTTTCTTGTACAAAGTGGT-3'

attP1: 5'-TACAGGTCACATAATACCATCTAAGTAGTTGATTCATAGTGACTGGATATG-TTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTA-ATATATTGATATTTATATCATTTTACGTTTCTCGTTCAGCTTTTTTGTAC-AAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACGAACA-GGTCATCATCAGTCAAAATAAAATCATTATTTG-3'

attP2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAAAT-TGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGAAC-GAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCAT-AAAAACAGACTACATAACTGTAAAACACAACATATCCAGTCACTATGA-ATCAACTACTTAGATGGTATTAGTGACCTGTA-3'

attR1: 5'-ACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAA-TATCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATAC-TGTAAAACACAACATATCCAGTCACTATG-3'

attR2: 5'-GCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTAT-GTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTT-ATATCATTTTACGTTTCTCGTTCAGCTTTCTTGTACAAAGTGGT-3'

attL1: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAAC-AAATTGATAAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAAA-GCAGGCT-3'

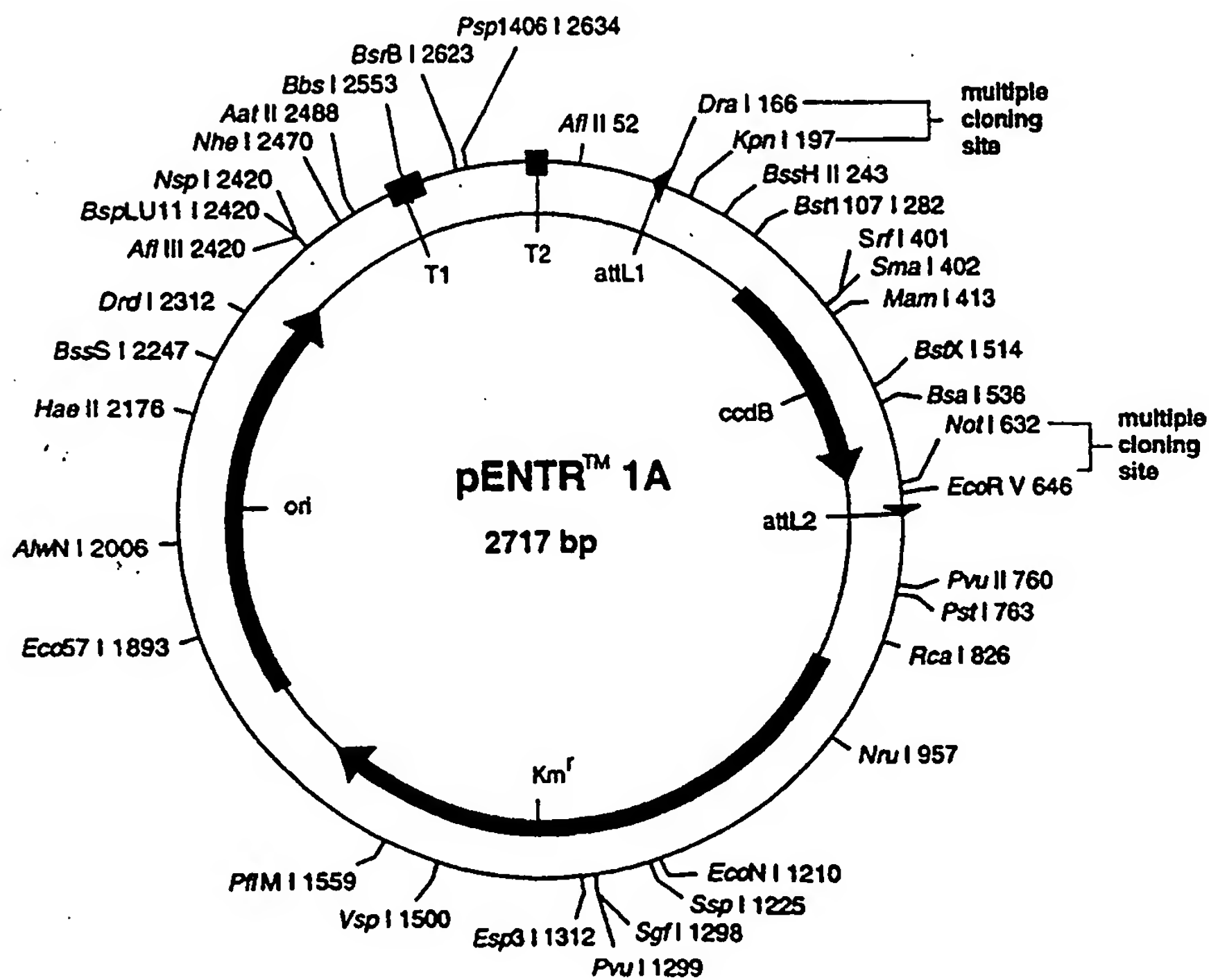
attL2: 5'-CAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCGTTGCAACAA-ATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTGGGT-3'

**Figure 9**

**Figure 10A: Cloning sites of the Entry Vector pENTR<sup>TM</sup>1A (reading frame A)**

ACT TTG TAC AAA AAA GCA GGC TTT AAA GGA ACC AAT TCA GTC GAC TGG ATC CGG TAC CGA ATT C  
 TGA AAC ATG TTT TTT CGT CCG AAA TTT CCT TGG TTA AGT CAG CTG ACC TAG GCC ATG GCT TAA G  
 thr leu tyr lys lys ala gly phe lys gly thr asn ser val asp trp ile arg tyr arg ile

EcoR I Not I Xho I EcoR V  
 --- ccdB gene --- G AAT TCG CCG CCG CAC TCG AGA TAT CTA GAC CCA GCT TTC TTG TAC AAA  
 C TTA AGC GCC GGC GTG AGC TCT ATA GAT CTG GGT CGA AAG AAC ATG TTT



## pENTR1A 2717 bp

Base Nos.	Gene Encoded
67..166	attL1
321..626	ccdB
655..754	attL2
877..1686	KmR
1791..2364	ori

```
1 CTGACGGATG GCCTTTTTGCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTAA AGGAACCAAT
181 TCAGTCGACT GGATCCGGTA CCGAATTCGC TTAATAAAG CCAGATAACA GTATGCGTAT
241 TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA
301 AAAAGAGGTG TGCTTCTAGA ATGCAGTTTA AGGTTTACAC CTATAAAAGA GAGAGCCGTT
361 ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC GCCCGGGCGA CGGATAGTGA
421 TCCCCCTGGC CAGTGACAGT CTGCTGTCAG ATAAAGTCTC CCGTGAACTT TACCCGGTGG
481 TGCATATCGG GGATGAAAGC TGGCGCATGA TGACCACCGA TATGGCCAGT GTGCCGGTCT
541 CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA AAATGACATC AAAAACGCCA
601 TTAACCTGAT GTTCTGGGGA ATATAGAATT CGCGGCCGCA CTCGAGATAT CTAGACCCAG
661 CTTTCTTGTA CAAAGTTGGC ATTATAAGAA AGCATTGCTT ATCAATTTGT TGCAACGAAC
721 AGGTCACAT CAGTCAAAAT AAAATCATT TTTGCCATCC AGCTGCAGCT CTGGCCCGTG
781 TCTCAAAATC TCTGATGTTA CATTGCACAA GATAAAAATA TATCATCATG AACATAAAAA
841 CTGTCTGCTT ACATAAACAG TAATACAAGG GGTGTTATGA GCCATATTCA ACGGGAAACG
901 TCGAGGCCGC GATTAAATTC CAACATGGAT GCTGATTTAT ATGGGTATAA ATGGGCTCGC
961 GATAATGTCG GGCAATCAGG TCGGACAATC TATCGCTTGT ATGGGAAGCC CGATGCGCCA
1021 GAGTTGTTTC TGAAACATGG CAAAGGTAGC GTTGCCAATG ATGTTACAGA TGAGATGGTC
1081 AGACTAAACT GGCTGACGGA ATTTATGCCT CTTCCGACCA TCAAGCATT TATCCGTACT
1141 CCTGATGATG CATGGTTACT CACCACTGCG ATCCCCGGAA AAACAGCATT CCAGGTATTA
1201 GAAGAATATC CTGATTCAGG TGAAAATATT GTTGATGCGC TGGCAGTGTC CCTGCGCCGG
1261 TTGCATTCGA TTCCTGTTTG TAATTGTCCT TTTAACAGCG ATCGCGTATT TCGTCTCGCT
1321 CAGGCGCAAT CACGAATGAA TAACGGTTTG GTTGATGCGA GTGATTTTGA TGACGAGCGT
1381 AATGGCTGGC CTGTTGAACA AGTCTGGAAG GAAATGCATA AACTTTTGCC ATTCTCACC
1441 GATTTCAGTC TCACTCATGG TGATTTCTCA CTTGATAACC TTATTTTGA CGAGGGGAAA
1501 TTAATAGGTT GTATTGATGT TGGACGAGTC GGAATCGCAG ACCGATACCA GGATCTTGCC
1561 ATCCTATGGA ACTGCCTCGG TGAGTTTCT CTTTCATTAC AGAAACGGCT TTTTCAAAAA
1621 TATGGTATTG ATAATCCTGA TATGAATAAA TTGCAGTTTC ATTGATGCT CGATGAGTTT
1681 TTCTAATCAG AATTGGTTAA TTGGTTGTAA CATTATTGAG ATTGGGCCCC GTTCCACTGA
1741 GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA
1801 ATCTGCTGCT TGCAAAACAA AAAACCACCG CTACCAGCGG TGGTTTGTTT GCCGGATCAA
1861 GAGCTACCAA CTCTTTTCC GAAGGTAAGT GGCTTCAGCA GAGCGCAGAT ACCAAATACT
1921 GTTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA
1981 TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT
2041 ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC AGCGGTCGGG CTGAACGGGG
2101 GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG
2161 CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA
2221 AGCGGCAGGG TCGGAACAGG AGAGCGCAG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT
2281 CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGACTTGAGC GTCGATTTTT GTGATGCTCG
2341 TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG CCTTTTACG GTTCTGGGCC
2401 TTTTGCTGGC CTTTGCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTC TGTGGATAAC
2461 CGTATTACCG CTAGCATGGA TCTCGGGGAC GTCTAACTAC TAAGCGAGAG TAGGGAACTG
2521 CCAGGCATCA AATAAAACGA AAGGCTCAGT CGGAAGACTG GGCTTTTCGT TTTATCTGTT
2581 GTTTGTCGGT GAACGCTCTC CTGAGTAGGA CAAATCCGCC GGGAGCGGAT TTGAACGTTG
2641 TGAAGCAACG GCCCGGAGGG TGGCGGGCAG GACGCCCGCC ATAAACTGCC AGGCATCAAA
2701 CTAAGCAGAA GGCCATC
```

FIGURE 10B

**Figure 11A: Cloning Sites of the Entry Vector pENTR2B (reading frame B)**

Int	attL1	EheI	XmnI	SalI	BamHI											
TTG	TAC	AAA	AAA	GCA	GGC	TGG	CSC	CGG	AAC	CAA	TTC	AGT	CGA	CTG	GAT	CCG
AAC	ATG	TTT	TTT	CGT	CCG	ACC	GCG	GCC	TTG	GTT	AAG	TCA	GCT	GAC	CTA	GGC
Leu	Tyr	Lys	Lys	Ala	Gly	Trp	Arg	Arg	Asn	Gln	Phe	Ser	Arg	Leu	Asp	Pro

KpnI	EcoRI	EcoRI	NotI	XhoI	EcoRV	XbaI										
GTA	CCG	AAT	TC-	ccdB	--G	AAT	TCG	CGG	CCG	CAC	TCG	AGA	TAT	CTA	GAC	CCA
CAT	GGC	TTA	AG			C	TTA	AGC	GCC	GGC	GTG	AGC	TCT	ATA	GAT	CTG
Val	Pro	Asn				Asn	Ser	Arg	Pro	His	Ser	Arg	Tyr	Leu	Asp	Pro

Int	attL2				
GCT	TTC	TTG	TAC	AAA	G
CGA	AAG	AAC	ATG	TTT	C
Ala	Phe	Leu	Tyr	Lys	

## pENTR2B 2718 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
322..627	ccdB
656..755	attL2
878..1687	KmR
1792..2365	ori

```
1 CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTGGCG CCGGAACCAA
181 TTCAGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA GCCAGATAAC AGTATGCGTA
241 TTTGCGCGCT GATTTTTCGCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC
301 AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA CCTATAAAAG AGAGAGCCGT
361 TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA CGCCCGGGCG ACGGATGGTG
421 ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT CCCGTGAACT TTACCCGGTG
481 GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG ATATGGCCAG TGTGCCGGTC
541 TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG AAAATGACAT CAAAAACGCC
601 ATTAACCTGA TGTTCTGGGG AATATAGAAT TCGCGGCCGC ACTCGAGATA TCTAGACCCA
661 GCTTTCCTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT TATCAATTTG TTGCAACGAA
721 CAGGTCACCTA TCAGTCAAAA TAAAATCATT ATTTGCCATC CAGCTGCAGC TCTGGCCCGT
781 GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAT ATATCATCAT GAACAATAAA
841 ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG AGCCATATTC AACGGGAAAC
901 GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA TATGGGTATA AATGGGCTCG
961 CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG TATGGGAAGC CCGATGCGCC
1021 AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT GATGTTACAG ATGAGATGGT
1081 CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC ATCAAGCATT TTATCCGTAC
1141 TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA AAAACAGCAT TCCAGGTATT
1201 AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTGATGCG CTGGCAGTGT TCCTGCGCCG
1261 GTTGCAATTCG ATTCCTGTTT GTAATTGTCC TTTTAACAGC GATCGCGTAT TTCGTCTCGC
1321 TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG AGTGATTTTG ATGACGAGCG
1381 TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT AAACTTTTGC CATTCTCACC
1441 GGATTCAGTC GTCACCTCATG GTGATTTCTC ACTTGATAAC CTTATTTTTG ACGAGGGGAA
1501 ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA GACCGATACC AGGATCTTGC
1561 CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA CAGAAACGGC TTTTTCAAAA
1621 ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT CATTTGATGC TCGATGAGTT
1681 TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA GATTGGGCCC CGTTCCACTG
1741 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT
1801 AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA
1861 AGAGCTACCA ACTCTTTTTT CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC
1921 TGTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC
1981 ATACCTCGCT CTGCTAATCC TGTTACCACT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT
2041 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG
2101 GGGTTCGTGC ACACAGCCCA GCTTGAGGCG AACGACCTAC ACCGAACTGA GATACCTACA
2161 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT
2221 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA
2281 TCTTTATAGT CCTGTCCGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC
2341 GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC
2401 CTTTTCGTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA
2461 CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAACTA CTAAGCGAGA GTAGGGAAC
2521 GCCAGGCATC AAATAAAACG AAAGGCTCAG TCGGAAGACT GGGCCTTTTCG TTTTATCTGT
2581 TGTTCGTGCG TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT
2641 GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC CAGGCATCAA
2701 ACTAAGCAGA AGGCCATC
```



**Figure 24: Cloning Sites of the Entry Vector pENTR3C (reading frame C)**

Int	attL1		DraI		XmnI		Sall		BamHI								
TTG	TAC	AAA	AAA	GCA	GGC	TCT	TTA	AAG	GAA	CCA	ATT	CAG	TCG	ACT	GGA	TCC	GGT
AAC	ATG	TTT	TTT	CGT	CCG	AGA	AAT	TTC	CTT	GGT	TAA	GTC	AGC	TGA	CCT	AGG	CCA
							↓			↓			↓		↓	↓	↓
Leu	Tyr	Lys	Lys	Ala	Gly	Ser	Leu	Lys	Glu	Pro	Ile	Gln	Ser	Thr	Gly	Ser	Gly

KpnI	EcoRI	PvuI		EcoRI	NotI		XhoI		EcoRV	XbaI						
ACC	GAA	TTC	GAT	CGC	--	ccdB	--G	AAT	TCG	CGG	CCG	CAC	TCG	AGA	TAT	CTA
TGG	CTT	AAG	CTA	GCG			C	TTA	AGC	GCC	GGC	GTG	AGC	TCT	ATA	GAT
			↓					↓		↓		↓	↓	↓	↓	↓
Thr	Glu	Phe						Asn	Ser	Arg	Pro	His	Ser	Arg	Tyr	Leu

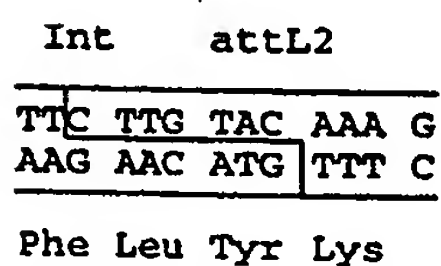
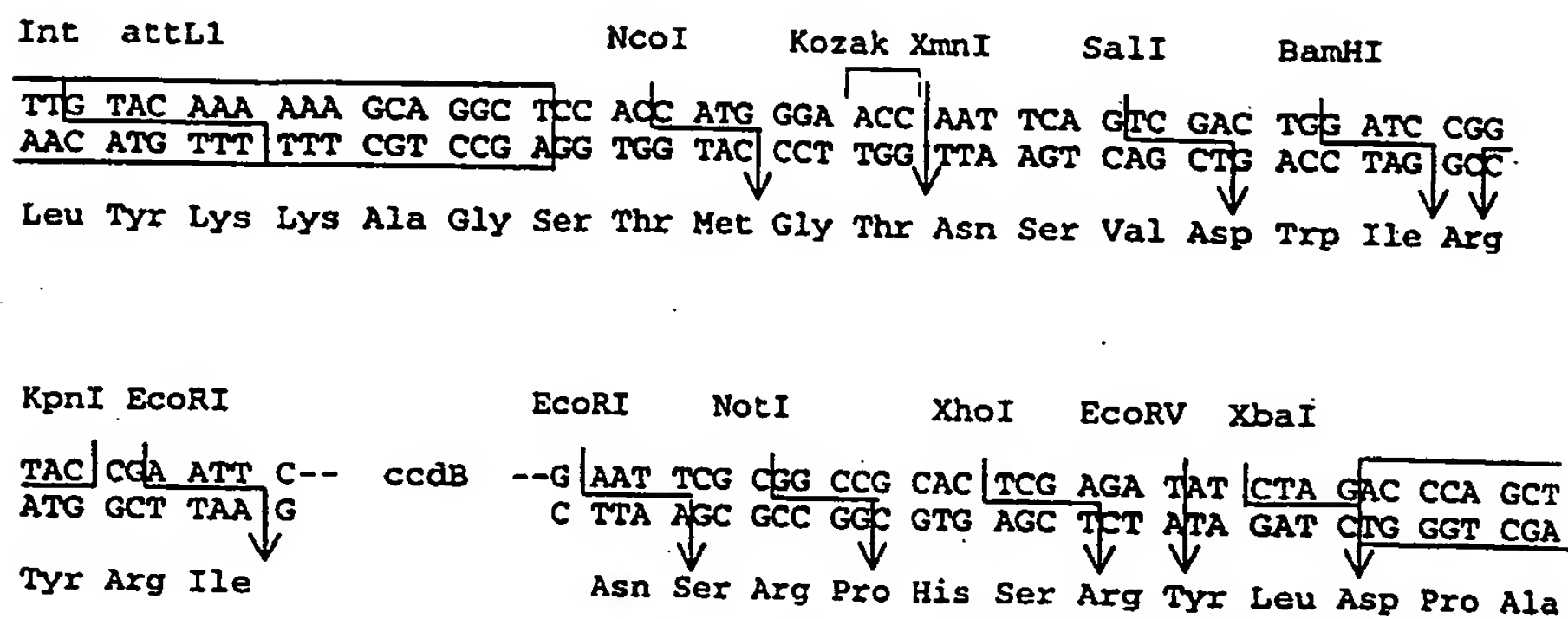
attL2	Int						
GAC	CCA	GCT	TTC	TTG	TAC	AAA	G
CTG	GGT	CGA	AAG	AAC	ATG	TTT	C
			↓				
Asp	Pro	Ala	Phe	Leu	Tyr	Lys	

## pENTR3C 2723 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
327..632	ccdB
661..760	attL2
883..1692	KmR
1797..2370	ori

```
1 CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTCTTT AAAGGAACCA
181 ATTCAGTCGA CTGGATCCGG TACCGAATTC GATCGCTTAC TAAAAGCCAG ATAACAGTAT
241 GCGTATTTGC GCGCTGATTT TTGCGGTATA AGAATATATA CTGATATGTA TACCCGAAGT
301 ATGTCAAAAA GAGGTGTGCT TCTAGAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA
361 GCCGTTATCG TCTGTTTGTG GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA
421 TGGTGATCCC CTTGGCCAGT GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC
481 CGGTGGTGCA TATCGGGGAT GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC
541 CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA
601 ACGCCATTAA CCTGATGTTT TGGGGAATAT AGAATTCGCG GCCGCACTCG AGATATCTAG
661 ACCCAGCTTT CTTGTACAAA GTTGGCATTG TAAGAAAGCA TTGCTTATCA ATTTGTTGCA
721 ACGAACAGGT CACTATCAGT CAAAATAAAA TCATTATTG CCATCCAGCT GCAGCTCTGG
781 CCCGTGTCTC AAAATCTCTG ATGTTACATT GCACAAGATA AAAATATATC ATCATGAACA
841 ATAAACTGT CTGCTTACAT AAACAGTAAT ACAAGGGGTG TTATGAGCCA TATTCAACGG
901 GAAACGTCGA GGCCGCGATT AAATCCAAAC ATGGATGCTG ATTTATATGG GTATAAATGG
961 GCTCGCGATA ATGTCGGGCA ATCAGGTGCG ACAATCTATC GCTTGTATGG GAAGCCCGAT
1021 GCGCCAGAGT TGTTTCTGAA ACATGGCAAA GGTAGCGTTG CCAATGATGT TACAGATGAG
1081 ATGGTCAGAC TAAACTGGCT GACGGAATTT ATGCCTCTTC CGACCATCAA GCATTTTATC
1141 CGTACTCCTG ATGATGCATG GTTACTCACC ACTGCGATCC CCGGAAAAAC AGCATTCCAG
1201 GTATTAGAAG AATATCCTGA TTCAGGTGAA AATATTGTTG ATGCGCTGGC AGTGTTCTCTG
1261 CGCCGGTTGC ATTCGATTCC TGTTTGTAAT TGTCCTTTTA ACAGCGATCG CGTATTTCTG
1321 CTCGCTCAGG CGCAATCACG AATGAATAAC GGTTTGGTTG ATGCGAGTGA TTTTGATGAC
1381 GAGCGTAATG GCTGGCCTGT TGAACAAGTC TGGAAAGAAA TGCATAAACT TTTGCCATTC
1441 TCACCCGATT CAGTCGTCAC TCATGGTGAT TTCTCACTTG ATAACCTTAT TTTTGACGAG
1501 GGGAAATTAA TAGGTTGTAT TGATGTTGGA CGAGTCGGAA TCGCAGACCG ATACCAGGAT
1561 CTTGCCATCC TATGGAACG CTCTGGTGAG TTTTCTCCTT CATTACAGAA ACGGCTTTTT
1621 CAAAAATATG GTATTGATAA TCCTGATATG AATAAATTGC AGTTTCATTT GATGCTCGAT
1681 GAGTTTTTCT AATCAGAATT GGTAAATTGG TTGTAACATT ATTCAGATTG GGCCCCGTTT
1741 CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG
1801 CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG
1861 GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA
1921 AATACTGTTT TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG
1981 CCTACATACC TCGCTCTGCT AATCCTGTGA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG
2041 TGTCTTACCG GGTGGAATC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA
2101 ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC
2161 CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT
2221 CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC
2281 TGGTATCTTT ATAGTCCGTG CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA
2341 TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC
2401 CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG
2461 GATAACCGTA TTACCGCTAG CATGGATCTC GGGGACGTCT AACTACTAAG CGAGAGTAGG
2521 GAACTGCCAG GCATCAAATA AAACGAAAGG CTCAGTCGGA AGACTGGGCC TTTCGTTTTA
2581 TCTGTTGTTT GTCGGTGAAC GCTCTCCTGA GTAGGACAAA TCCGCCGGA GCGGATTTGA
2641 ACGTTGTGAA GCAACGGCCC GGAGGGTGGC GGGCAGGACG CCCGCCATAA ACTGCCAGGC
2701 ATCAAATAA GCAGAAGGCC ATC
```

FIGURE 12B

**Figure 13A: Cloning Sites of the Entry Vector pENTR4 :**

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## pENTR4 2720 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
67..166	attL1
324..629	ccdB
658..757	attL2
880..1689	KmR
1794..2367	ori

```
1 CTGACGGATG GCCTTTTTGC GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAT GCCAACTTTG TACAAAAAAG CAGGCTCCAC CATGGGAACC
181 AATTCAGTCG ACTGGATCCG GTACCGAATT CGCTTACTAA AAGCCAGATA ACAGTATGCG
241 TATTTGCGCG CTGATTTTTG CGGTATAAGA ATATATACTG ATATGTATAC CCGAAGTATG
301 TCAAAAAGAG GTGTGCTTCT AGAATGCAGT TTAAGGTTTA CACCTATAAA AGAGAGAGCC
361 GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG CGACGGATGG
421 TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG
481 TGGTGCAATAT CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG
541 TCTCCGTTAT CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG
601 CCATTAACCT GATGTTCTGG GGAATATAGA ATTGCGGGCC GCACTCGAGA TATCTAGACC
661 CAGCTTTCTT GTACAAAGTT GGCATTATAA GAAAGCATTG CTTATCAATT TGTGCAACG
721 AACAGGTCAC TATCAGTCAA AATAAAATCA TTATTTGCCA TCCAGCTGCA GCTCTGGCCC
781 GTGTCTCAAA ATCTCTGATG TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA
841 AAAGTGTCTG CTTACATAAA CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA
901 ACGTCGAGGC CGCGATTAAA TTCCAACATG GATGCTGATT TATATGGGTA TAAATGGGCT
961 CGCGATAATG TCGGGCAATC AGGTGCGACA ATCTATCGCT TGTATGGGAA GCCCGATGCG
1021 CCAGAGTTGT TTCTGAAACA TGGCAAAGGT AGCGTTGCCA ATGATGTTAC AGATGAGATG
1081 GTCAGACTAA ACTGGCTGAC GGAATTTATG CCTCTCCGA CCATCAAGCA TTTTATCCGT
1141 ACTCCTGGTG ATGCATGGTT ACTCACCCT GCGATCCCCG GAAAAACAGC ATTCCAGGTA
1201 TTAGAAGAAT ATCCTGATTC AGGTGAAAAT ATTGTTGATG CGCTGGCAGT GTTCCTGCGC
1261 CGGTTGCATT CGATTCTGTG TTGTAATTGT CTTTTTAACA GCGATCGCGT ATTTCTGCTC
1321 GCTCAGGCGC AATCACGAAT GAATAACGGT TTGGTTGATG CGAGTGATTT TGATGACGAG
1381 CGTAATGGCT GGCCTGTTGA ACAAGTCTGG AAAGAAATGC ATAACTTTT GCCATTCTCA
1441 CCGGATTCAG TCGTCACTCA TGGTGATTTT TCACTTGATA ACCTTATTTT TGACGAGGGG
1501 AAATTAATAG GTTGTAATTG TGTGACGCA GTCGGAATCG CAGACCGATA CCAGGATCTT
1561 GCCATCCTAT GGAAGTGCCT CGGTGAGTTT TCTCCTTCAT TACAGAAACG GCTTTTTTCAA
1621 AAATATGGTA TTGATAATCC TGATATGAAT AAATTGCAGT TTCATTTGAT GCTCGATGAG
1681 TTTTCTAAT CAGAATTGGT TAATTGGTTG TAACATTATT CAGATTGGGC CCCGTTCCAC
1741 TGAGCGTCAG ACCCCGTAAG AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC
1801 GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT
1861 CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT
1921 ACTGTTCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACC GCCT
1981 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGCGCA TAAGTCGTGT
2041 CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG
2101 GGGGGTTTCGT GCACACAGCC CAGCTTGAGG CGAACGACCT ACACCGAACT GAGATACCTA
2161 CAGCGTGAGC TATGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG
2221 GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
2281 TATCTTTATA GTCTGTGCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC
2341 TCGTCAGGGG GCGGAGCCT ATGGAAAAAC GCCAGCAACG CGGCCTTTTT ACGGTTCTCTG
2401 GCCTTTTGCT GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCCTGA TTCTGTGGAT
2461 AACCGTATTA CCGCTAGCAT GGATCTCGGG GACGTCTAAC TACTAAGCGA GAGTAGGGAA
2521 CTGCCAGGCA TCAAAATAAA CGAAAGGCTC AGTCGGAAGA CTGGGCCTTT CGTTTTATCT
2581 GTTGTGTTGTC GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG GATTTGAACG
2641 TTGTGAAGCA ACGGCCCGGA GGGTGGCGGG CAGGACGCCC GCCATAAACT GCCAGGCATC
2701 AAATAAGCA GAAGGCCATC
```

FIGURE 13B



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## pENTR5 2720 bp

Location (Base Nos.)			Gene Encoded			
67..166			attL1			
324..629			ccdB			
658..757			attL2			
880..1689			KmR			
1794..2367			ori			
1	CTGACGGATG	GCCTTTTTTGC	GTTTCTACAA	ACTCTTCCTG	TTAGTTAGTT	ACTTAAGCTC
61	GGGCCCCAAA	TAATGATTTT	ATTTTGACTG	ATAGTGACCT	GTTTCGTTGCA	ACAAATTGAT
121	AAGCAATGCT	TTTTTATAAT	GCCAACCTTG	TACAAAAAAG	CAGGCTTTTCA	TATGGGAACC
181	AATTCAGTCG	ACTGGATCCG	GTACCGAATT	CGCTTACTAA	AAGCCAGATA	ACAGTATGCG
241	TATTTGCGCG	CTGATTTTTG	CGGTATAAGA	ATATATACTG	ATATGTATAC	CCGAAGTATG
301	TCAAAAAGAG	GTGTGCTTCT	AGAATGCAGT	TTAAGGTTTA	CACCTATAAA	AGAGAGAGCC
361	GTTATCGTCT	GTTTGTGGAT	GTACAGAGTG	ATATTATTGA	CACGCCCGGG	CGACGGATGG
421	TGATCCCCCT	GGCCAGTGCA	CGTCTGCTGT	CAGATAAAGT	CTCCCGTGAA	CTTTACCCGG
481	TGGTGCATAT	CGGGGATGAA	AGCTGGCGCA	TGATGACCAC	CGATATGGCC	AGTGTGCCGG
541	TCTCCGTTAT	CGGGGAAGAA	GTGGCTGATC	TCAGCCACCG	CGAAAATGAC	ATCAAAAACG
601	CCATTAACCT	GATGTTCTGG	GGAATATAGA	ATTCGCGGCC	GCACTCGAGA	TATCTAGACC
661	CAGCTTTCTT	GTACAAAGTT	GGCATTATAA	GAAAGCATTG	CTTATCAATT	TGTTGCAACG
721	AACAGGTCAC	TATCAGTCAA	AATAAAATCA	TTATTTGCCA	TCCAGCTGCA	GCTCTGGCCC
781	GTGTCTCAAA	ATCTCTGATG	TTACATTGCA	CAAGATAAAA	ATATATCATC	ATGAACAATA
841	AAACTGTCTG	CTTACATAAA	CAGTAATACA	AGGGGTGTTA	TGAGCCATAT	TCAACGGGAA
901	ACGTCGAGGC	CGCGATTAAA	TTCCAACATG	GATGCTGATT	TATATGGGTA	TAAATGGGCT
961	CGCGATAATG	TCGGGCAATC	AGGTGCGACA	ATCTATCGCT	TGTATGGGAA	GCCCGATGCG
1021	CCAGAGTTGT	TTCTGAAACA	TGGCAAAGGT	AGCGTTGCCA	ATGATGTTAC	AGATGAGATG
1081	GTCAGACTAA	ACTGGCTGAC	GGAATTTATG	CCTCTTCCGA	CCATCAAGCA	TTTTATCCGT
1141	ACTCCTGATG	ATGCATGGTT	ACTCACCCT	GCGATCCCCG	GAAAAACAGC	ATTCCAGGTA
1201	TTAGAAGAAT	ATCCTGATTC	AGGTGAAAAT	ATTGTTGATG	CGCTGGCAGT	GTTCTGCGC
1261	CGGTTGCATT	CGATTCTCTG	TTGTAATTGT	CCTTTTAACA	GCGATCGCGT	ATTTCTGCTC
1321	GCTCAGGCGC	AATCACGAAT	GAATAACGGT	TTGGTTGATG	CGAGTGATTT	TGATGACGAG
1381	CGTAATGGCT	GGCCTGTTGA	ACAAGTCTGG	AAAGAAATGC	ATAAACTTTT	GCCATTCTCA
1441	CCGGATTTCAG	TCGTCACTCA	TGGTGATTTT	TCATTGATA	ACCTTATTTT	TGACGAGGGG
1501	AAATTAATAG	GTTGTATTGA	TGTTGGACGA	GTCGGAATCG	CAGACCGATA	CCAGGATCTT
1561	GCCATCCTAT	GGAAGTGCCT	CGGTGAGTTT	TCTCCTTCAT	TACAGAAACG	GCTTTTTCAT
1621	AAATATGGTA	TTGATAATCC	TGATATGAAT	AAATTGCAGT	TTCATTTGAT	GCTCGATGAG
1681	TTTTTCTAAT	CAGAATTGGT	TAATTGGTTG	TAACATTATT	CAGATTGGGC	CCCGTTCCAC
1741	TGAGCGTCAG	ACCCCGTAGA	AAAGATCAAA	GGATCTTCTT	GAGATCCTTT	TTTTCTGCGC
1801	GTAATCTGCT	GCTTGCAAAC	AAAAAAACCA	CCGCTACCAG	CGGTGGTTTG	TTTGCCGGAT
1861	CAAGAGCTAC	CAACTCTTTT	TCCGAAGGTA	ACTGGCTTCA	GCAGAGCGCA	GATACCAAAT
1921	ACTGTTCTTC	TAGTGTAGCC	GTAGTTAGGC	CACCACTTCA	AGAACTCTGT	AGCACCGCCT
1981	ACATACCTCG	CTCTGCTAAT	CCTGTTACCA	GTGGCTGCTG	CCAGTGCGCA	TAAGTCGTGT
2041	CTTACCGGGT	TGGACTCAAG	ACGATAGTTA	CCGGATAAGG	CGCAGCGGTC	GGGCTGAACG
2101	GGGGGTTTCG	GCACACAGCC	CAGCTTGGAG	CGAACGACCT	ACACCGAACT	GAGATACCTA
2161	CAGCGTGAGC	TATGAGAAAG	CGCCACGCTT	CCCGAAGGGA	GAAAGGCGGA	CAGGTATCCG
2221	GTAAGCGGCA	GGGTCGGAAC	AGGAGAGCGC	ACGAGGGAGC	TTCCAGGGGG	AAACGCCTGG
2281	TATCTTTATA	GTCCTGTCTG	GTTTCGCCAC	CTCTGACTTG	AGCGTCGATT	TTTGTGATGC
2341	TCGTCAAGGG	GGCGGAGCCT	ATGGAAAAAC	GCCAGCAACG	CGGCCTTTTT	ACGGTTCCTG
2401	GCCTTTTGCT	GGCCTTTTGC	TCACATGTTT	TTTCCTGCGT	TATCCCCTGA	TTCTGTGGAT
2461	AACCGTATTA	CCGCTAGCAT	GGATCTCGGG	GACGTCTAAC	TACTAAGCGA	GAGTAGGGAA
2521	CTGCCAGGCA	TCGAATAAAA	CGAAAGGCTC	AGTCGGAAGA	CTGGGCCTTT	CGTTTTATCT
2581	GTTGTTTGTC	GGTGAACGCT	CTCCTGAGTA	GGACAAATCC	GCCGGGAGCG	GATTTGAACG
2641	TTGTGAAGCA	ACGGCCCGGA	GGGTGGCGGG	CAGGACGCCC	GCCATAAACT	GCCAGGCATC
2701	AAACTAAGCA	GAAGGCCATC				

Figure 14B



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Figure 15A: Cloning sites of the Entry Vector pENTR6

Int	attL1				Sph I		Kpn I	Xmn I	Sal I				
5'	ctg	tac	aaa	aaa	gca	ggc	tyc	atg	cga	acc	aat	tca	gtc
3'	aac	atg	tct	ttt	cgt	ccg	acc	tac	get	tgg	tta	agt	cag
	Leu	Tyr	Lys	Lys	Ala	Gly	Cys	Met	Arg	Thr	Asn	Ser	Val

BamH I	Kpn I		EcoR I			EcoR I
gac	tgg	atc	cgg	tac	cga	att
cgg	acc	tag	gcp	atg	get	taa
	Asp	Trp	Ile	Arg	Tyr	Arg

--- Death --- (cod8) --- tct taa ggc

Not	Xho I	EcoR I	Xba I	Int		attL2
ggc	cgc	act	cga	gat	atc	tag
ccg	ggg	tga	gct	cta	tag	atc

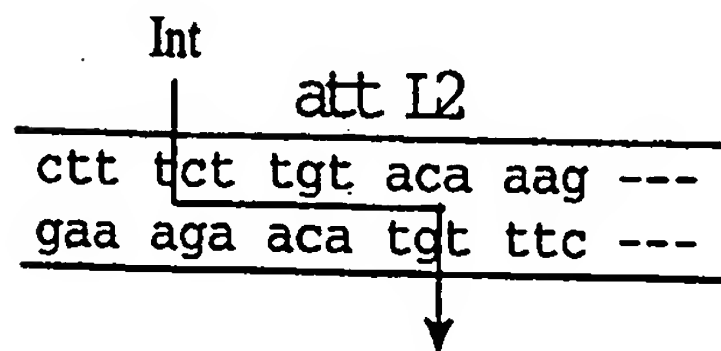
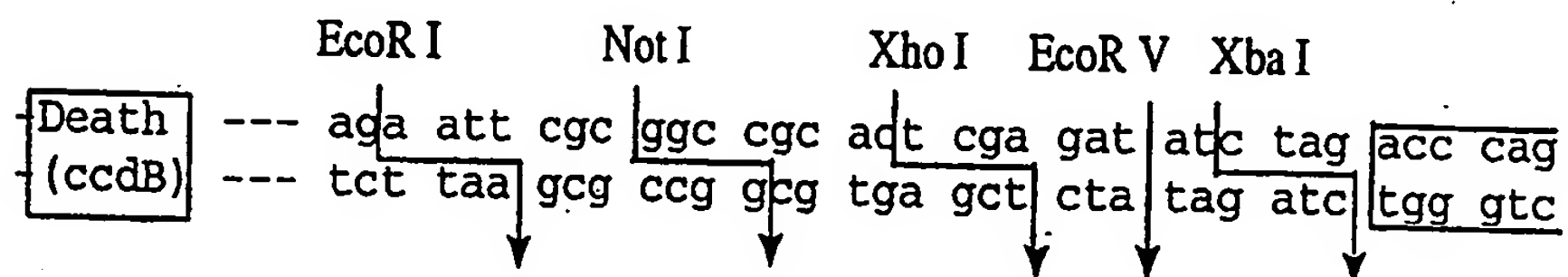
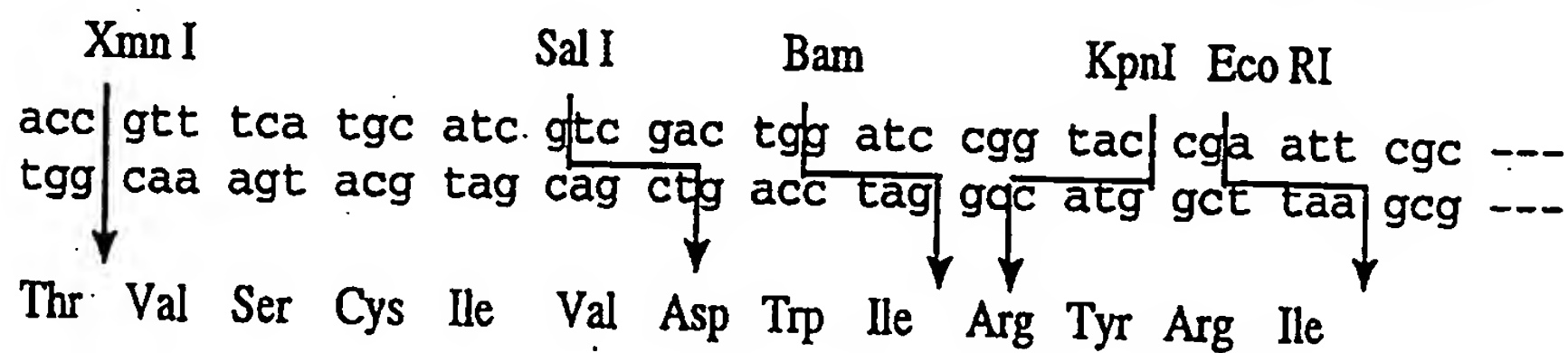
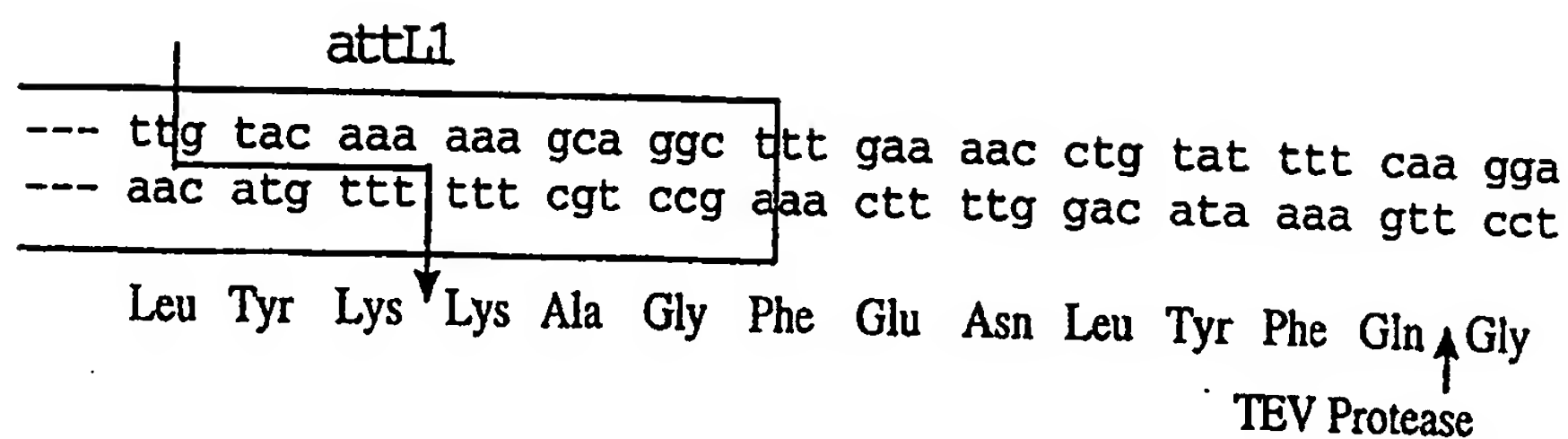
acc cag ctt tct tgt aga aag ---  
tgg gtc gaa aga aca tgt ttc ---

## pENTR6 2717 bp

Location (Base Nos.)		Gene Encoded	
67..166		attL1	
321..626		ccdB	
655..754		attL2	
877..1686		KmR	
1791..2364		ori	
1	CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC		
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT		
121	AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTGCAT GCGAACCAAT		
181	TCAGTCGACT GGATCCGGTA CCGAATTCGC TTAATAAAAG CCAGATAACA GTATGCGTAT		
241	TTGCGCGCTG ATTTTTCGCG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA		
301	AAAAGAGGTG TGCTTCTAGA ATGCAGTTTA AGGTTTACAC CTATAAAAGA GAGAGCCGTT		
361	ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC GCCCGGGCGA CGGATGGTGA		
421	TCCCCCTGGC CAGTGCACGT CTGCTGTCAG ATAAAGTCTC CCGTGAACTT TACCCGGTGG		
481	TGCATATCGG GGATGAAAGC TGGCGCATGA TGACCACCGA TATGGCCAGT GTGCCGGTCT		
541	CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA AAATGACATC AAAAACGCCA		
601	TTAACCTGAT GTTCTGGGGA ATATAGAATT CGCGGCCGCA CTCGAGATAT CTAGACCCAG		
661	CTTTCCTGTA CAAAGTTGGC ATTATAAGAA AGCATTGCTT ATCAATTTGT TGCAACGAAC		
721	AGGTCACAT CAGTCAAAAT AAAATCATTA TTTGCCATCC AGCTGCAGCT CTGGCCCGTG		
781	TCTCAAAATC TCTGATGTTA CATTGCACAA GATAAAAATA TATCATCATG AACATAAAAA		
841	CTGTCTGCTT ACATAAACAG TAATACAAGG GGTGTTATGA GCCATATTCA ACGGGAAACG		
901	TCGAGGCCGC GATTAAATTC CAACATGGAT GCTGATTTAT ATGGGTATAA ATGGGCTCGC		
961	GATAATGTCG GGCAATCAGG TGCGACAATC TATCGCTTGT ATGGGAAGCC CGATGCGCCA		
1021	GAGTTGTTTC TGAAACATGG CAAAGGTAGC GTTGCCAATG ATGTTACAGA TGAGATGGTC		
1081	AGACTAAACT GGCTGACGGA ATTTATGCCT CTTCCGACCA TCAAGCATT TATCCGTACT		
1141	CCTGATGATG CATGGTTACT CACCACTGCG ATCCCCGGA AAACAGCATT CCAGGTATTA		
1201	GAAGAATATC CTGATTCAGG TGAAAAATAT GTTGATGCGC TGGCAGTGTT CCTGCGCCGG		
1261	TTGCATTCTG TTCCTGTTTG TAATTGTCCT TTTAACAGCG ATCGCGTATT TCGTCTCGCT		
1321	CAGGCGCAAT CACGAATGAA TAACGGTTTG GTTGATGCGA GTGATTTTGA TGACGAGCGT		
1381	AATGGCTGGC CTGTTGAACA AGTCTGGAAA GAAATGCATA AACTTTTGCC ATTCTCACCG		
1441	GATTCAGTCG TCACTCATGG TGATTTCTCA CTTGATAACC TTATTTTGA CGAGGGGAAA		
1501	TTAATAGGTT GTATTGATGT TGGACGAGTC GGAATCGCAG ACCGATACCA GGATCTTGCC		
1561	ATCCTATGGA ACTGCCTCGG TGAGTTTCT CTTTCATTAC AGAAACGGCT TTTTCAAAAA		
1621	TATGGTATTG ATAATCCTGA TATGAATAAA TTGCAGTTTC ATTTGATGCT CGATGAGTTT		
1681	TTCTAATCAG AATTGGTTAA TTGGTTGTAA CATTATTCAG ATTGGGCCCC GTTCCACTGA		
1741	GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA		
1801	ATCTGCTGCT TGCAACAAA AAAACCACCG CTACCAGCGG TGGTTTGTTT GCCGGATCAA		
1861	GAGCTACCAA CTCTTTTTCC GAAGGTAACT GGCTTCAGCA GAGCGCAGAT ACCAAATACT		
1921	GTTCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA ACTCTGTAGC ACCGCCTACA		
1981	TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA GTGGCGATAA GTCGTGTCTT		
2041	ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC AGCGGTCGGG CTGAACGGGG		
2101	GGTTCGTGCA CACAGCCAG CTTGGAGCGA ACGACCTACA CCGAACTGAG ATACCTACAG		
2161	CGTGAGCTAT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA AGGCGGACAG GTATCCGGTA		
2221	AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT		
2281	CTTTATAGTC CTGTCGGGTT TCGCCACCTC TGAATTGAGC GTCGATTTTT GTGATGCTCG		
2341	TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG CCTTTTACG GTTCCTGGCC		
2401	TTTTGCTGGC CTTTGTCTCA CATGTTCTTT CCTGCGTTAT CCCCTGATTG TGTGGATAAC		
2461	CGTATTACCG CTAGCATGGA TCTCGGGGAC GTCTAACTAC TAAGCGAGAG TAGGGAACTG		
2521	CCAGGCATCA AATAAAACGA AAGGCTCAGT CGGAAGACTG GGCCTTTCGT TTTATCTGTT		
2581	GTTTGTCCGT GAACGCTCTC CTGAGTAGGA CAAATCCGCC GGGAGCGGAT TTGAACGTTG		
2641	TGAAGCAACG GCCCGGAGGG TGGCGGGCAG GACGCCCGCC ATAAACTGCC AGGCATCAAA		
2701	CTAAGCAGAA GGCCATC		

22/12/00

Figure 16A: Cloning sites of the Entry Vector pENTR7



## pENTR7 2738 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
342..647	ccdB
676..775	attL2
898..1707	KmR
1812..2385	ori

```
1 CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT
181 TTTCAAGGAA CCGTTTCATG CATCGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA
241 GCCAGATAAC AGTATGCGTA TTTGCGCGCT GATTTTTCG GTATAAGAAT ATATACTGAT
301 ATGTATACCC GAAGTATGTC AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA
361 CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA
421 CGCCCGGGCG ACGGATAGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT
481 CCCGTGAACT TTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG
541 ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG
601 AAAATGACAT CAAAACGCC ATTAACCTGA TGTCTGGGG AATATAGAAT TCGCGGCCGC
661 ACTCGAGATA TCTAGACCCA GCTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT
721 TATCAATTTG TTGCAACGAA CAGGTCACCTA TCAGTCAAAA TAAAATCATT ATTTGCCATC
781 CAGCTGCAGC TCTGGCCCGT GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAT
841 ATATCATCAT GAACAATAAA ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG
901 AGCCATATTC AACGGGAAAC GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA
961 TATGGGTATA AATGGGCTCG CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG
1021 TATGGGAAGC CCGATGCGCC AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT
1081 GATGTTACAG ATGAGATGGT CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC
1141 ATCAAGCATT TTATCCGTAC TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA
1201 AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTCAG GTGAAAATAT TGTGATGCG
1261 CTGGCAGTGT TCCTGCGCCG GTTGCAATCG ATTCTGTTT GTAATTGTCC TTTTAACAGC
1321 GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG
1381 AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT
1441 AAACTTTTGC CATTCTCACC GGATTCAGTC GTCACTCATG GTGATTTCTC ACTTGATAAC
1501 CTTATTTTTC ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA
1561 GACCGATACC AGGATCTTGC CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA
1621 CAGAAACGGC TTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT
1681 CATTGATGTC TCGATGAGTT TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA
1741 GATTGGGCCC CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA
1801 GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG
1861 GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC
1921 AGAGCGCAGA TACCAATAC TGTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG
1981 AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC
2041 AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG
2101 CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC
2161 ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA
2221 AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT
2281 CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG
2341 CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG
2401 GCCTTTTTTAC GGTTCCTGGC CTTTGTCTGG CTTTGTCTC ACATGTTCTT TCCTGCGTTA
2461 TCCCCTGATT CTGTGGATAA CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAACTA
2521 CTAAGCGAGA GTAGGGAAC GCCAGGCATC AAATAAAACG AAAGGCTCAG TCGGAAGACT
2581 GGGCCTTTTCG TTTTATCTGT TGTGTCGGG TGAACGCTCT CCTGAGTAGG ACAAATCCGC
2641 CGGGAGCGGA TTTGAACGTT GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCGC
2701 CATAAACTGC CAGGCATCAA ACTAAGCAGA AGGCCATC
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Figure 17A: Cloning Sites of the *ENTY* Vector, pENTR8

Int      attL

```

  /--| 5'g tac aaa aaa gca ggc ttt|gaa aac ctg tat ttt caa gga
  /--| 3'g acg acg atc|ttt cgt ccg aaa|ctt ttg gac ata aaa gtt cct

```

Leu Tyr Lys Lys Ala Gly Phe Glu Asn Leu Tyr Phe Gln Gly

TEV Protease

NotI    AclI      SalI      BsmHI      KpnI    EcoRI

```

  acc atg gac cta gtc gac tgg atc cgg tac|cga att cgc ---
  tgg tac|ctg|gat cag cgg acc tag|gcb atg gct taa|gcb ---

```

Thr Met Asp Leu Val Asp Trp Ile Arg Tyr Arg Ile

EcoRI    NotI      XhoI    EcoRV    XhoI      attL

```

  Death --- aga att cgc|ggc cgc act cga gat|acc tag|acc cag
  --- tct taa|gcb ccg gcb tga gct|cta|tag atc|tgg gtc

```

Int

```

  ctt tct|tgt|aca|aac|---
  gaa aga aca tgt ttc|---

```

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## pENTR8 2735 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
339..644	ccdB
673..772	attL2
895..1704	KmR
1809..2382	ori

```

1 CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT
181 TTTCAAGGAA CCATGGACCT AGTCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC
241 AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG
301 TATACCCGAA GTATGTCAAA AAGAGGTGTG CTTCTAGAAT GCAGTTTAAG GTTTACACCT
361 ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC
421 CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGTCT GCTGTCAGAT AAAGTCTCCC
481 GTGAACTTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA
541 TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA
601 ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCG CGGCCGCACT
661 CGAGATATCT AGACCCAGCT TTCTTGTAACA AAGTTGGCAT TATAAGAAAG CATTGCTTAT
721 CAATTTGTTG CAACGAACAG GTCACTATCA GTCAAAATAA AATCATTATT TGCCATCCAG
781 CTGCAGCTCT GGCCCGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA
841 TCATCATGAA CAATAAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC
901 CATATTCAAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT
961 GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGTAT
1021 GGGAAAGCCCG ATGCGCCAGA GTTGTTCCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT
1081 GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC
1141 AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCCAGAAAA
1201 ACAGCATTC CAGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG
1261 GCAGTGTCCT TGCGCCGGTT GCATTGCAAT CCTGTTTGTA ATTGTCTCTT TAACAGCGAT
1321 CGCGTATTTT GTCTCGCTCA GCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT
1381 GATTTTGATG ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA
1441 CTTTGTCCAT TCTCACCAGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT
1501 ATTTTGTGAC AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC
1561 CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG
1621 AAACGGCTTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT
1681 TTGATGCTCG ATGAGTTTTC CTAATCAGAA TTGGTTAATT GGTTGTAACA TTATTGAGAT
1741 TGGGCCCCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT
1801 CCTTTTTCCT TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG
1861 GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACCTG CTTGAGCAGA
1921 GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC
1981 TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
2041 GCGGATAAGT CGTGTCCTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG
2101 CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCAGCT TGGAGCGAAC GACCTACACC
2161 GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG
2221 GCGGACAGGT ATCCGGTAAG CCGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA
2281 GGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
2341 CGATTTTGTG GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACCGGGCC
2401 TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TCGGTTATCC
2461 CCTGATTCTG TGGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAATACTA
2521 AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAAACGAAA GGCTCAGTCG GAAGACTGGG
2581 CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCGCG
2641 GAGCGGATTT GAACGTTGTG AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCCGCCAT
2701 AAACGCGCAG GCATCAAACT AAGCAGAAGG CCATC

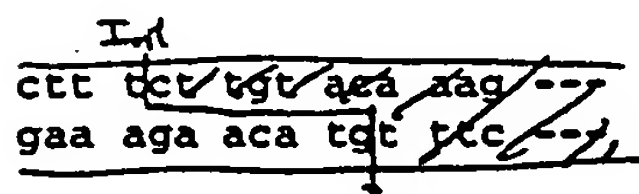
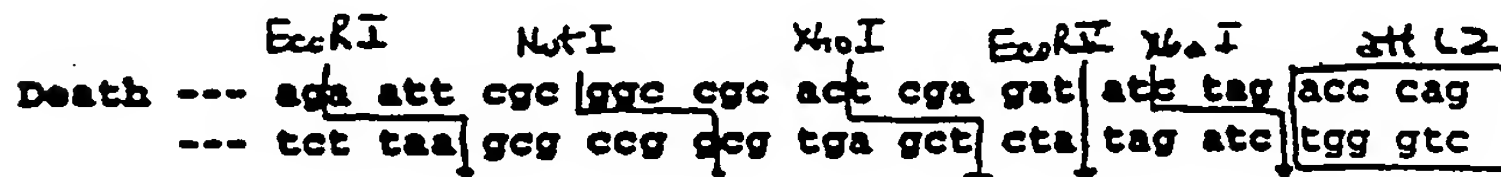
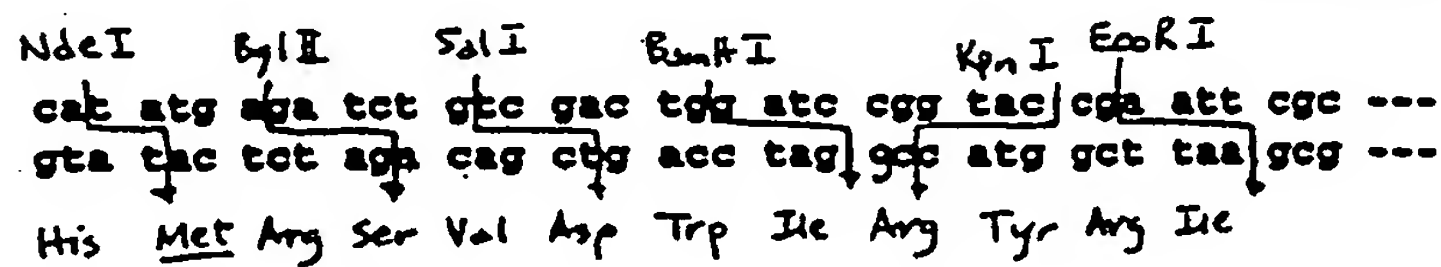
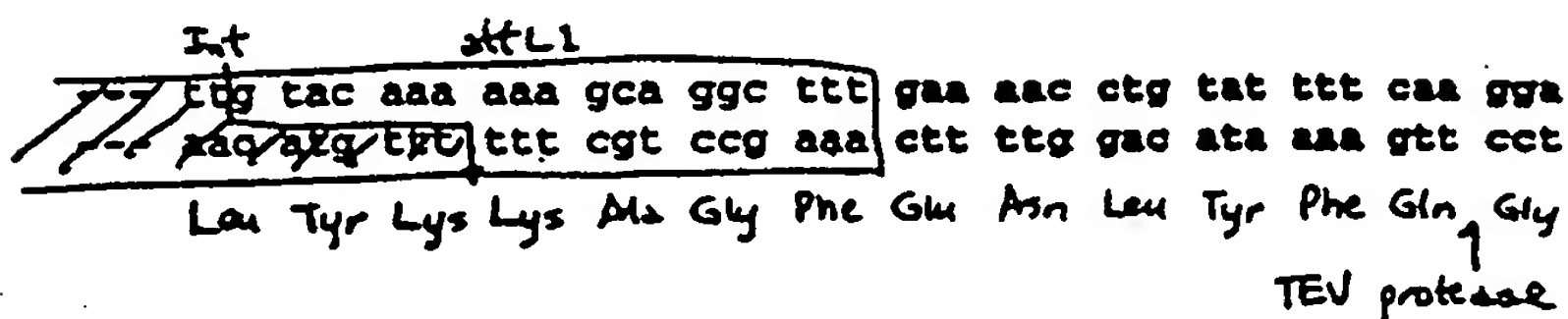
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FIGURE 17B



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Figure 18A: Cloning sites of the ENTRY Vector pENTRY



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## pENTR9 2735 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
339..644	ccdB
673..772	attL2
895..1704	KmR
1809..2382	ori

```
1 CTGACGGATG GCCTTTTTCG GTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTTGA AAACCTGTAT
181 TTTCAAGGAC ATATGAGATC TGTCGACTGG ATCCGGTACC GAATTCGCTT ACTAAAAGCC
241 AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG
301 TATACCCGAA GTATGTCAAA AAGAGGTGTG CTTCTAGAAT GCAGTTTAAG GTTTACACCT
361 ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA GAGTGATATT ATTGACACGC
421 CCGGGCGACG GATAGTGATC CCCCTGGCCA GTGCACGTCT GCTGTCAGAT AAAGTCTCCC
481 GTGAACTTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG GCGCATGATG ACCACCGATA
541 TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC TGATCTCAGC CACCGCGAAA
601 ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT ATAGAATTCTG CGGCCGCACT
661 CGAGATATCT AGACCCAGCT TTCTTGATCA AAGTTGGCAT TATAAGAAAG CATTGCTTAT
721 CAATTTGTTG CAACGAACAG GTCATATCA GTCAAAATAA AATCATTATT TGCCATCCAG
781 CTGCAGCTCT GGCCCGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA
841 TCATCATGAA CAATAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTATGAGC
901 CATATTCAAC GGGAAACGTC GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT
961 GGGTATAAAT GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTTGAT
1021 GGGAAAGCCG ATGCGCCAGA GTTGTCTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT
1081 GTTACAGATG AGATGGTCAG ACTAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC
1141 AAGCATTTTA TCCGTACTCC TGATGATGCA TGGTTACTCA CCACTGCGAT CCCCAGGAAA
1201 ACAGCATTCC AGGTATTAGA AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG
1261 GCAGTGTCCT TGCGCCGGTT GCATTCGATT CCTGTTTGTA ATTGTCCTTT TAACAGCGAT
1321 CGCGTATTTT GTCTCGCTCA GGCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT
1381 GATTTTGATG ACGAGCGTAA TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATAAA
1441 CTTTGGCCAT TCTCACCAGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT
1501 ATTTTGGACG AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC
1561 CGATACCAGG ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG
1621 AAACGGCTTT TTCAAAAATA TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT
1681 TTGATGCTCG ATGAGTTTCT CTAATCAGAA TTGGTTAATT GGTGTAACA TTATTCAGAT
1741 TGGGCCCCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT
1801 CCTTTTTCCT TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT ACCAGCGGTG
1861 GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAAGTGG CTTTCAAGCA
1921 GCGCAGATAC CAAATACTGT TCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC
1981 TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
2041 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG
2101 CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCAGCT TGGAGCGAAC GACCTACACC
2161 GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG
2221 GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA
2281 GGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
2341 CGATTTTGTG GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC
2401 TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTC TGCGTTATCC
2461 CCTGATTCTG TGGATAACCG TATTACCGCT AGCATGGATC TCGGGGACGT CTAACACTA
2521 AGCGAGAGTA GGGAACTGCC AGGCATCAAA TAAACGAAA GGCTCAGTCG GAAGACTGGG
2581 CCTTTCGTTT TATCTGTTGT TTGTCGGTGA ACGCTCTCCT GAGTAGGACA AATCCGCCGG
2641 GAGCGGATTT GAACGTTGTG AAGCAACGGC CCGGAGGGTG GCGGGCAGGA CGCCGCCCAT
2701 AAACGCGCAG GCATCAAACT AAGCAGAAGG CCATC
```

Figure 18B

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Figure 19A: Cloning sites of the ENTRY Vector pENTRY10

Int                      attL1                      S.D.                      -12                      Nde

--- tgg tac aaa aaa gca ggc ttc gaa cta agg aaa tac tta cgt  
 --- aag atg ttc ttt cgt ccg aag ctt gat tcc ttt atg aat gta  
 Leu Tyr Lys Lys Ala Gly Phe Glu Leu Arg Lys Tyr Leu His

K3 Xba                      Sal                      Bam                      Kpn                      EcoRI

atg gga acc aat tca gtc gac tgg atc cgg tac cga att cgc ---  
 tac cct tgg tta agt cag cgg acc tag gcp atg gct taa gcg ---  
 Met Gly Thr Asn Ser Val Asp Trp Ile Arg Tyr Arg Ile

EcoRI                      Not                      Xho                      EcoRII                      Xba                      attL2

Death --- aga att cgc ggc cgc act cga gat atc tag acc cag  
 (ccdB)--- tct taa gcg ccg gcg tga gct cta tag atc tgg gtc

Int

ctt tgg tgg aca aag aag  
 gaa aga aca tgc ttc ---

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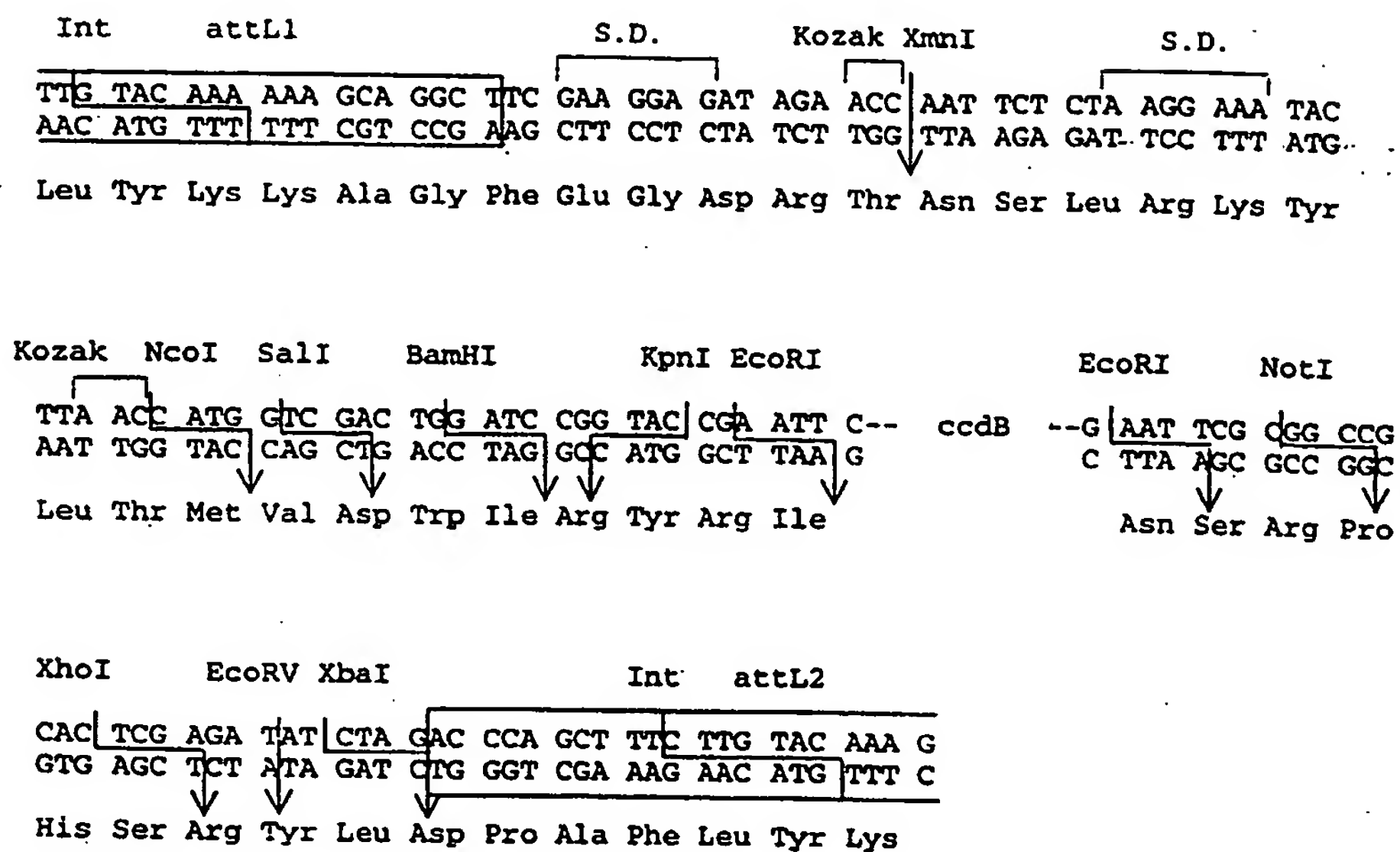
## pENTR10 2738 bp

Location (Base Nos.)	Gene Encoded
67..166	attL1
342..647	ccdB
676..775	attL2
898..1707	KmR
1812..2385	ori

```
1 CTGACGGATG GCCTTTTTCG GTTTCTACAA ACTCTTCCTG TTAGTTAGTT ACTTAAGCTC
61 GGGCCCCAAA TAATGATTTT ATTTTGACTG ATAGTGACCT GTTCGTTGCA ACAAATTGAT
121 AAGCAATGCT TTTTATAAAT GCCAACTTTG TACAAAAAAG CAGGCTTCGA ACTAAGGAAA
181 TACTTACATA TGGGAACCAA TTCAGTCGAC TGGATCCGGT ACCGAATTCG CTTACTAAAA
241 GCCAGATAAC AGTATGCGTA TTTGCGCGCT GATTTTTCG GTATAAGAAT ATATACTGAT
301 ATGTATACCC GAAGTATGTC AAAAAGAGGT GTGCTTCTAG AATGCAGTTT AAGGTTTACA
361 CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATTGACA
421 CGCCCGGGCG ACGGATGGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAAGTCT
481 CCCGTGAACT TTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG
541 ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGAAGAAGT GGCTGATCTC AGCCACCGCG
601 AAAATGACAT CAAAAACGCC ATTAACCTGA TGTTCCTGGG AATATAGAAT TCGCGGCCGC
661 ACTCGAGATA TCTAGACCCA GCTTCTTGT ACAAAGTTGG CATTATAAGA AAGCATTGCT
721 TATCAATTTG TTGCAACGAA CAGGTCAC TAAGTCAAAA TAAATCATT ATTTGCCATC
781 CAGCTGCAGC TCTGGCCCGT GTCTCAAAAT CTCTGATGTT ACATTGCACA AGATAAAAAT
841 ATATCATCAT GAACAATAAA ACTGTCTGCT TACATAAACA GTAATACAAG GGGTGTATG
901 AGCCATATTC AACGGGAAAC GTCGAGGCCG CGATTAAATT CCAACATGGA TGCTGATTTA
961 TATGGGTATA AATGGGCTCG CGATAATGTC GGGCAATCAG GTGCGACAAT CTATCGCTTG
1021 TATGGGAAGC CCGATGCGCC AGAGTTGTTT CTGAAACATG GCAAAGGTAG CGTTGCCAAT
1081 GATGTTACAG ATGAGATGGT CAGACTAAAC TGGCTGACGG AATTTATGCC TCTTCCGACC
1141 ATCAAGCATT TTATCCGTAC TCCTGATGAT GCATGGTTAC TCACCACTGC GATCCCCGGA
1201 AAAACAGCAT TCCAGGTATT AGAAGAATAT CCTGATTGAG GTGAAAATAT TGTGATGCG
1261 CTGGCAGTGT TCCTGCGCCG GTTGCAATCG ATTCCTGTTT GTAATTGTCC TTTTAACAGC
1321 GATCGCGTAT TTCGTCTCGC TCAGGCGCAA TCACGAATGA ATAACGGTTT GGTGATGCG
1381 AGTGATTTTG ATGACGAGCG TAATGGCTGG CCTGTTGAAC AAGTCTGGAA AGAAATGCAT
1441 AAACTTTTGC CATTCTCACC GGATTGAGT GTCACTCATG GTGATTTCTC ACTTGATAAC
1501 CTTATTTTTG ACGAGGGGAA ATTAATAGGT TGTATTGATG TTGGACGAGT CGGAATCGCA
1561 GACCGATACC AGGATCTTGC CATCCTATGG AACTGCCTCG GTGAGTTTTC TCCTTCATTA
1621 CAGAAACGGC TTTTTCAAAA ATATGGTATT GATAATCCTG ATATGAATAA ATTGCAGTTT
1681 CATTGATGTC TCGATGAGTT TTTCTAATCA GAATTGGTTA ATTGGTTGTA ACATTATTCA
1741 GATTGGGCCC CGTTCCACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA
1801 GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC GCTACCAGCG
1861 GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC
1921 AGAGCGCAGA TACCAAATAC TGTTCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG
1981 AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT GGCTGCTGCC
2041 AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG
2101 CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC
2161 ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA
2221 AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT
2281 CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTGCGGT TTCGCCACCT CTGACTTGAG
2341 CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG
2401 GCCTTTTTAC GGTTCCTGGC CTTTGTCTGG CTTTTGCTC ACATGTTCTT TCCTGCGTTA
2461 TCCCCTGATT CTGTGGATAA CCGTATTACC GCTAGCATGG ATCTCGGGGA CGTCTAACTA
2521 CTAAGCGAGA GTAGGGAACG GCCAGGCATC GAATAAACG AAAGGCTCAG TCGGAAGACT
2581 GGGCCTTTTC TTTTATCTGT TGTGTGTCGG TGAACGCTCT CCTGAGTAGG ACAAATCCGC
2641 CGGGAGCGGA TTTGAACGTT GTGAAGCAAC GGCCCGGAGG GTGGCGGGCA GGACGCCCGC
2701 CATAAACTGC CAGGCATCAA ACTAAGCAGA AGGCCATC
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FIGURE 19B

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**Figure 20A: Cloning Sites of the Entry Vector pENTR1.1**

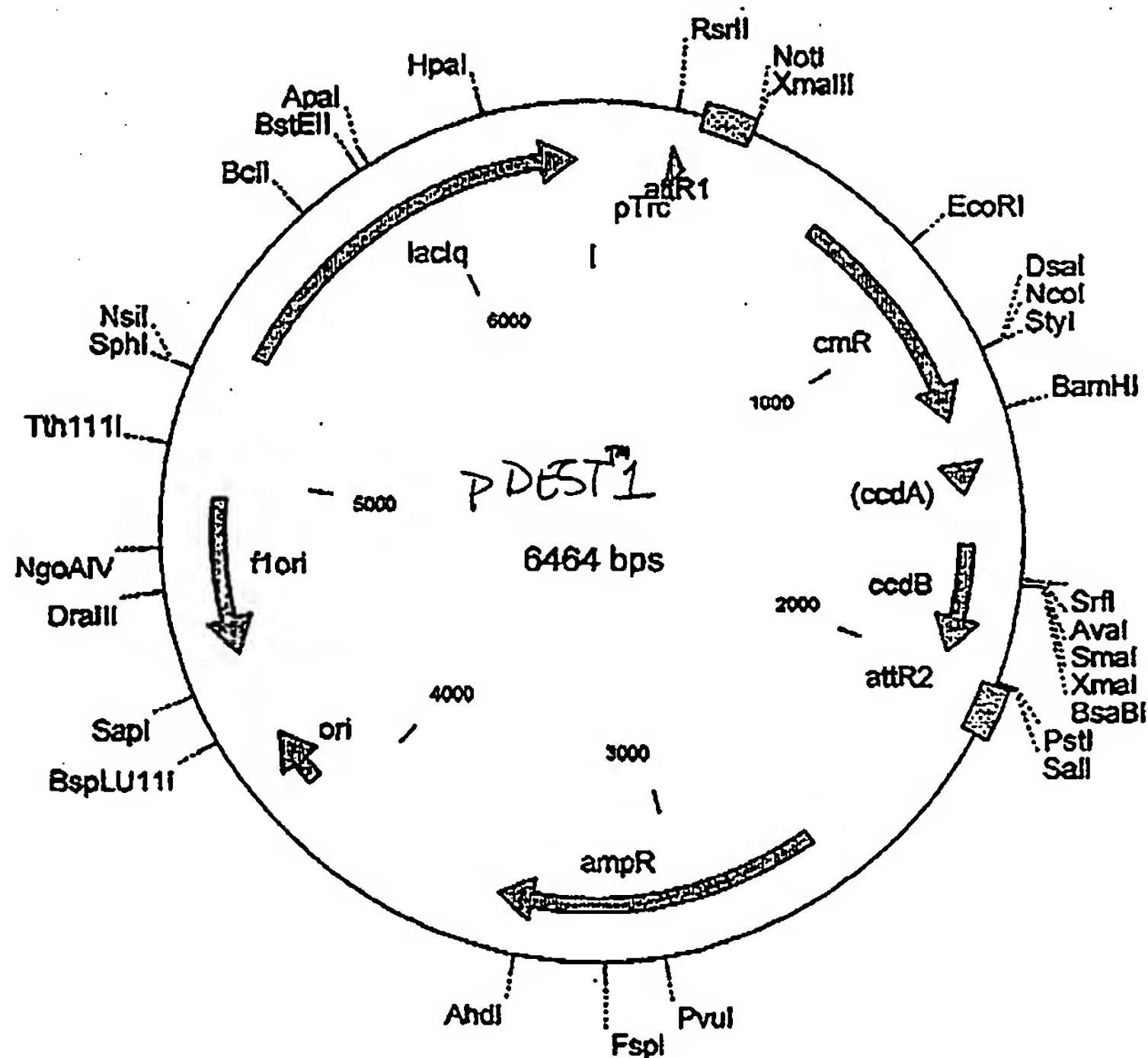
## pENTR11 2744 bp (rotated to position 2578)

Location (Base Nos.)		Gene Encoded	
67..166		attL1	
348..653		ccdB	
683..781		attL2	
904..1713		KmR	
1818..2391		ori	
1	CTGACGGATG GCCTTTTTGC GTTCTACAA	ACTCTTCCTG	TTAGTTAGTT ACTTAAGCTC
61	GGGCCCCAAA TAATGATTTT ATTTTGACTG	ATAGTGACCT	GTTTCGTTGCA ACAAATTGAT
121	AAGCAATGCT TTTTATAAT GCCAACTTTG	TACAAAAAAG	CAGGCTTCGA AGGAGATAGA
181	ACCAATTCTC TAAGGAAATA CTTAACCATG	GTCGACTGGA	TCCGGTACCG AATTTCGCTTA
241	CTAAAAGCCA GATAACAGTA TGCCTATTTG	CGCGCTGATT	TTTGCGGTAT AAGAATATAT
301	ACTGATATGT ATACCCGAAG TATGTCAAAA	AGAGGTGTGC	TTCTAGAATG CAGTTTAAGG
361	TTTACACCTA TAAAAGAGAG AGCCGTTATC	GTCTGTTTGT	GGATGTACAG AGTGATATTA
421	TTGACACGCC CGGGCGACGG ATAGTGATCC	CCCTGGCCAG	TGCACGTCTG CTGTCAGATA
481	AAGTCTCCCG TGAACTTTAC CCGGTGGTGC	ATATCGGGGA	TGAAAGCTGG CGCATGATGA
541	CCACCGATAT GGCCAGTGTG CCGGTCTCCG	TTATCGGGGA	AGAAGTGGCT GATCTCAGCC
601	ACCGCGAAAA TGACATCAAA AACGCCATTA	ACCTGATGTT	CTGGGGAATA TAGAATTTCG
661	GGCCGCACTC GAGATATCTA GACCCAGCTT	TCTTGTTACA	AGTTGGCATT ATAAGAAAGC
721	ATTGCTTATC AATTTGTTGC AACGAACAGG	TCACTATCAG	TCAAAATAAA ATCATTATTT
781	GCCATCCAGC TGCAGCTCTG GCGGTGTCT	CAAAATCTCT	GATGTTACAT TGCACAAGAT
841	AAAAATATAT CATCATGAAC AATAAACTG	TCTGCTTACA	TAAACAGTAA TACAAGGGGT
901	GTTATGAGCC ATATTCAACG GGAAACGTCG	AGGCCGCGAT	TAAATTCCAA CATGGATGCT
961	GATTTATATG GGTATAAATG GGCTCGCGAT	AATGTCGGGC	AATCAGGTGC GACAATCTAT
1021	CGCTTGATG GGAAGCCCGA TGCGCCAGAG	TTGTTTCTGA	AACATGGCAA AGGTAGCGTT
1081	GCCAATGATG TTACAGATGA GATGGTCAGA	CTAAACTGGC	TGACGGAATT TATGCCTCTT
1141	CCGACCATCA AGCATTATAT CCGTACTCCT	GATGATGCAT	GGTTACTCAC CACTGCGATC
1201	CCCGGAAAAA CAGCATTCCA GGTATTAGAA	GAATATCCTG	ATTCAGGTGA AAATATTGTT
1261	GATGCGCTGG CAGTGTTCTT GCGCCGGTTG	CATTTCGATT	CTGTTTGTA TGTCTCTTTT
1321	AACAGCGATC GCGTATTTCT TCTCGCTCAG	GCGCAATCAC	GAATGAATAA CGGTTTGTTT
1381	GATGCGAGTG ATTTTGATGA CGAGCGTAAT	GGCTGGCCTG	TTGAACAAGT CTGGAAAGAA
1441	ATGCATAAAC TTTTGCCATT CTCACCGGAT	TCAGTCGTCA	CTCATGGTGA TTTCTCACTT
1501	GATAACCTTA TTTTGACGA GGGGAAATTA	ATAGGTTGTA	TTGATGTTGG ACGAGTCGGA
1561	ATCGCAGACC GATACCAGGA TCTTGCCATC	CTATGGAAC	GCCTCGGTGA GTTTTCTCCT
1621	TCATTACAGA AACGGCTTTT TCAAAAATAT	GGTATTGATA	ATCCTGATAT GAATAAATTG
1681	CAGTTTCATT TGATGCTCGA TGAGTTTTTC	TAATCAGAAT	TGGTTAATTG GTTGTAACAT
1741	TATTCAGATT GGGCCCCGTT CCACTGAGCG	TCAGACCCCG	TAGAAAAGAT CAAAGGATCT
1801	TCTTGAGATC CTTTTTTTCT GCGCGTAATC	TGCTGCTTGC	AAACAAAAAA ACCACCGCTA
1861	CCAGCGGTGG TTTGTTTGCC GGATCAAGAG	CTACCAACTC	TTTTTCCGAA GGTAAGTGGC
1921	TTCAAGAACT CGTAGCACC GCCTACATAC	CTCGCTCTGC	TAATCCTGTT ACCAGTGGCT
1981	GCTGCCAGTG GCGATAAGTC GTGTCTTACC	GGGTTGGACT	CAAGACGATA GTTACCGGAT
2041	AAGGCGCAGC GGTCCGGGCTG AACGGGGGGT	TCGTGCACAC	AGCCCAGCTT GGAGCGAACG
2101	ACCTACACCG AACTGAGATA CCTACAGCGT	GAGCTATGAG	AAAGCGCCAC GCTTCCCGAA
2161	GGGAGAAAGG CGGACAGGTA TCCGGTAAGC	GGCAGGGTCG	GAACAGGAGA GCGCACGAGG
2221	GAGCTTCCAG GGGGAAACGC CTGGTATCTT	TATAGTCCTG	TCGGGTTTCG CCACCTCTGA
2281	CTTGAGCGTC GATTTTGTG ATGCTCGTCA	GGGGGGCGGA	GCCTATGGAA AAACGCCAGC
2341	AACGCGGCCT TTTTACGGTT CCTGGCCCTT	TGCTGGCCTT	TTGCTCACAT GTTCTTTCTT
2401	GCGTTATCCC CTGATTCTGT GGATAACCGT	ATTACCGCTA	GCATGGATCT CGGGGACGTC
2461	TAACACTAA GCGAGAGTAG GGAAGTCCA	GGCATCAAAT	AAAACGAAAG GCTCAGTCGG
2521	AAGACTGGGC CTTTCGTTTT ATCTGTGTTT	TGTCGGTGAA	CGCTCTCCTG AGTAGGACAA
2581	ATCCGCCGGG AGCGGATTTG AACGTTGTGA	AGCAACGGCC	CGGAGGGTGG CGGGCAGGAC
2641	GCCCCCATA AACTGCCAGG CATCAAACTA	AGCAGAAGGC	CATC



**Figure 2/A: pDEST1 Native Protein Expression in E. coli**

1     <sup>-35</sup>     <sup>Tre promoter</sup>     <sup>-10</sup>     <sup>MANA</sup>  
 atgagctggt gacaattaat catccggctc gataatgtg tggattgtg agcggataac  
 tactcgacha ctgttaatta gtaggccgag catattacac accttaacac tcgcctattg  
 61     aatttcacac aggaacaga caggtatagg atcacaagtt ~~tgtagaada agctgaagga~~  
        ttaaagtgtg tcctttgtct gtccatatcc taggttcaa ~~acatgttct tcgacttct~~



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## pDEST1 6464 bp

Location (Base Nos.)	Gene Encoded
216..257	Trc promoter
397..273	attR1
647..1306	CmR
1426..1510	inactivated ccdA
1648..1953	ccdB
1994..2118	attR2
2598..3503	ampR
4104..4264	ori
4504..4941	flori (f1 intergenic region)
5340..6420	lacIq

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1 GTTTGACAGC TTATCATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC
61 GGAAGCTGTG GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC
121 GCACTCCCGT TCTGGATAAT GTTTTTTGCG CCGACATCAT AACGGTTCTG GCAAATATTC
181 TGAAATGAGC TGTTGACAAT TAATCATCCG GTCCGTATAA TCTGTGGAAT TGTGAGCGGG
241 ATAACAATTT CATCGCGAGG TACCAAGCTA TCACAAGTTT GTACAAAAAA GCTGAACGAG
301 AAACGTAAAA TGATATAAAT ATCAATATAT TAAATTAGAT TTTGCATAAA AAACAGACTA
361 CATAATACTG TAAAACACAA CATATCCAGT CACTATGGCG GCCGCTAAGT TGGCAGCATC
421 ACCCGACGCA CTTTGCGCCG AATAAATACC TGTGACGGAA GATCACTTCG CAGAATAAAT
481 AAATCCTGGT GTCCCTGTTG ATACCGGGAA GCCCTGGGCC AACTTTTGCG GAAAATGAGA
541 CGTTGATCGG CACGTAAGAG GTTCCAACCT TCACCATAAT GAAATAAGAT CACTACCGGG
601 CGTATTTTTT GAGTTATCGA GATTTTCAGG AGCTAAGGAA GCTAAAATGG AGAAAAAAT
661 CACTGGATAT ACCACCGTTG ATATATCCCA ATGGCATCGT AAAGAACATT TTGAGGCATT
721 TCAGTCAGTT GCTCAATGTA CCTATAACCA GACCGTTCAG CTGGATATTA CGGCCTTTTT
781 AAAGACCGTA AAGAAAAATA AGCACAAGTT TTATCCGGCC TTTATTCACA TTCTTGCCCG
841 CCTGATGAAT GCTCATCCGG AATTCCGTAT GGCAATGAAA GACGGTGAGC TGGTGATATG
901 GGATAGTGTT CACCCTTGTT ACACCGTTTT CCATGAGCAA ACTGAAACGT TTTCATCGCT
961 CTGGAGTGAA TACCACGACG ATTTCCGGCA GTTTCTACAC ATATATTCGC AAGATGTGGC
1021 GTGTTACGGT GAAAACCTGG CCTATTTCCC TAAAGGGTTT ATTGAGAATA TGTTTTTCGT
1081 CTCAGCCAAT CCCTGGGTGA GTTTCACCAG TTTTGATTTA AACGTGGCCA ATATGGACAA
1141 CTTCTTCGCC CCCGTTTTCA CCATGGGCAA ATATTATACG CAAGGCGACA AGGTGCTGAT
1201 GCCGCTGGCG ATTCAGGTTT ATCATGCCGT CTGTGATGGC TTCCATGTCG GCAGAATGCT
1261 TAATGAATTA CAACAGTACT GCGATGAGTG GCAGGGCGGG GCGTAAACGC GTGGATCCGG
1321 CTTACTAAAA GCCAGATAAC AGTATGCGTA TTTGCGCGCT GATTTTTGCG GTATAAGAAT
1381 ATATACTGAT ATGTATACCC GAAGTATGTC AAAAAGAGGT GTGCTATGAA GCAGCGTATT
1441 ACAGTGACAG TTGACAGCGA CAGCTATCAG TTGCTCAAGG CATATATGAT GTCAATATCT
1501 CCGGTCTGGT AAGCACAACC ATGCAGAAAT AAGCCCGTCG TCTGCGTGCC GAACGCTGGA
1561 AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG TCGCCCGGTT TATTGAAATG AACGGCTCTT
1621 TTGCTGACGA GAACAGGGAC TGGTGAAATG CAGTTTAAAG TTTACACCTA TAAAAGAGAG
1681 AGCCGTTATC GTCTGTTTGT GGATGTACAG AGTGATATTA TTGACACGCC CGGGCGACGG
1741 ATGGTGATCC CCCTGGCCAG TGCACGCTCT CTGTCAGATA AAGTCTCCCG TGAACCTTAC
1801 CCGGTGGTGC ATATCGGGGA TGAAAGCTGG CGCATGATGA CCACCGATAT GGCCAGTGTG
1861 CCGGTCTCCG TTATCGGGGA AGAAGTGGCT GATCTCAGCC ACCGCGAAAA TGACATCAAA
1921 AACGCCATTA ACCTGATGTT CTGGGGAATA TAAATGTCAG GCTCCCTTAT ACACAGCCAG
1981 TCTGCAGGTC GACCATAGTG ACTGGATATG TTGTGTTTTA CAGTATTATG TAGTCTGTTT
2041 TTTATGCAAA ATCTAATTTA ATATATTGAT ATTTATATCA TTTTACGTTT CTCGTTGAGC
2101 TTTCTTGATC AAAGTGGTGA TAGCTTGGCT GTTTTGCGCG ATGAGAGAAG ATTTTCAGCC
2161 TGATACAGAT TAAATCAGAA CGCAGAAGCG GTCTGATAAA ACAGAATTG CCTGGCGGCA
2221 GTAGCGCGGT GGTCCCACCT GACCCCATGC CGAACTCAGA AGTGAAACGC CGTAGCGCCG
2281 ATGGTAGTGT GGGGTCTCCC CATGCGAGAG TAGGGAAGTG CCAGGCATCA AATAAAACGA
2341 AAGGCTCAGT CGAAAGACTG GGCCTTTCGT TTTATCTGTT GTTTGTCGGT GAACGCTCTC
2401 CTGAGTAGGA CAAATCCGCC GGGAGCGGAT TTGAACGTTG CGAAGCAACG GCCCGGAGGG
2461 TGGCGGGCAG GACGCCCGCC ATAAACTGCC AGGCATCAAA TTAAGCAGAA GGCCATCCTG
2521 ACGGATGGCC TTTTTCGCTT TCTACAAACT CTTTTTGTTT ATTTTCTAA ATACATTCAA-

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FIGURE 2/B

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2581 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAAGGA  
2641 AGAGTATGAG TATTCAACAT TTCCGTGTCT CCCTTATTCC CTTTTTTGCG GCATTTTGCC  
2701 TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG  
2761 GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGASTTTTC  
2821 GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGCGCGGTAT  
2881 TATCCCGTGT TGACGCCGGG CAAGAGCAAC TCGGTGCGCG CATACTAT TCTCAGAATG  
2941 ACTTGGTTGA GTACTACCA GTACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG  
3001 AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA  
3061 CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAAGTC  
3121 GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA  
3181 CGATGCCTAC AGCAATGGCA ACAACGTTGC GCAAACTATT AACTGGCGAA CTACTTACTC  
3241 TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC  
3301 TGCCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG  
3361 GGTCTCGCG TATCATTGCA GCACTGGGCG CAGATGGTAA GCCCTCCCGT ATCGTAGTTA  
3421 TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG  
3481 GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTAATCATAT ATAATTTAGA  
3541 TTGATTTAAA ACTTCATTTT TAATTTAAA GGATCTAGGT GAAGATCCTT TTTGATAATC  
3601 TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA  
3661 AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAACAA  
3721 AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC  
3781 CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT  
3841 AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCTAC ATACCTCGCT CTGCTAATCC  
3901 TGTTACCAGT GGCTGTGCTC AGTGCGGATA AGTCGTGTCT TACCGGGTG GACTCAAGAC  
3961 GATAGTTACC GGATAAGGCG CAGCGGTGCG GCTGAACGGG GGGTTCGTGC ACACAGCCCA  
4021 GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG  
4081 CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG  
4141 GAGAGCGCAC GAGGGAGCTT CCAGGGGGA ACGCCGTGTA TCTTTATAGT CCTGTGCGGT  
4201 TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT  
4261 GGAAAAACGC CAGCAACGCG GCCTTTTTTAC GGTTCTTGGC CTTTGTGCTG CTTTTGCTC  
4321 ACATGTTCTT TCCTGCGTTA TCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT  
4381 GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG  
4441 CGGAAGAGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT TCACACCGCA  
4501 TAATTTTGTT AAAATTGCGG TTAATTTTTT GTTAAATCAG CTCATTTTTT AACCAATAGG  
4561 CCGAAATCGG CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTG  
4621 TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAAGCTGGA CTCCAACGTC AAAGGGCGAA  
4681 AAACCGTCTA TCAGGGCGAT GGCCCACTAC GTGAACCATC ACCCTAATCA AGTTTTTTGG  
4741 GGTGAGGTG CCGTAAAGCA CTAAATCGGA ACCCTAAAGG GAGCCCCGA TTTAGAGCTT  
4801 GACGGGGAAA GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG  
4861 CTAGGGCGCT GGCAAGTGTA GCGGTACGCG TGCGCGTAAC CACCACACC GCCGCGCTTA  
4921 ATGCGCCGCT ACAGGGCGCG TCCATTCGCC ATTCAGGCTG CTATGGTGCA CTCTCAGTAC  
4981 AATCTGCTCT GATGCCGCAT AGTTAAGCCA GTACCAGTCA CGTAGCGATA TCGGAGTGTA  
5041 TACACTCCGC TATCGCTACG TGACTGGGTC ATGGCTGCGC CCCGACACC GCCAACACC  
5101 GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC  
5161 GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG  
5221 CAGATCAATT CGCGCGCGAA GGCGAAGCGG CATGCATTTA CGTTGACACC ATCGAATGGT  
5281 GCAAAACCTT TCGCGGTATG GCATGATAGC GCCCGGAAGA GAGTCAATTC AGGGTGGTGA  
5341 ATGTGAAACC AGTAACGTTA TACGATGTCG CAGAGTATGC CCGTGTCTCT TATCAGACCG  
5401 TTTCCCGCGT GGTGAACGAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGAAG  
5461 CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACG GCGGGCAAAC  
5521 AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCG TCGCAAATTG  
5581 TCGCGCGGAT TAAATCTCGC GCCGATCAAC TGGGTGCCAG CGTGGTGGTG TCGATGGTAG  
5641 AACGAAGCGG CGTCGAAGCC TGTAAGCGG CCGTGACAA TCTTCTCGCG CAACGCGTCA  
5701 GTGGGCTGAT CATTAATAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT  
5761 GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC AACAGTATTA  
5821 TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGAGCA TCTGGTCGCA TTGGGTCAAC  
5881 AGCAAATCGC GCTGTAGCG GGCCCATTA GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG  
5941 GCTGGCATAA ATATCTCACT CGCAATCAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT  
6001 GGAGTGCCAT GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA-

Figure 21C

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6061 CTGCGATGCT GGTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT  
6121 CCGGGCTGCG CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT  
6181 CATGTTATAT CCCGCCGTTA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA  
6241 GCGTGGACCG CTTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC  
6301 CCGTCTCACT GGTGAAAAGA AAAACCACCC TGGCACCCAA TACGCAAACC GCCTCTCCCC  
6361 GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC  
6421 AGTGAGCGCA ACGCAATTAA TGTGAGTTAG CGCGAATTGA TCTG

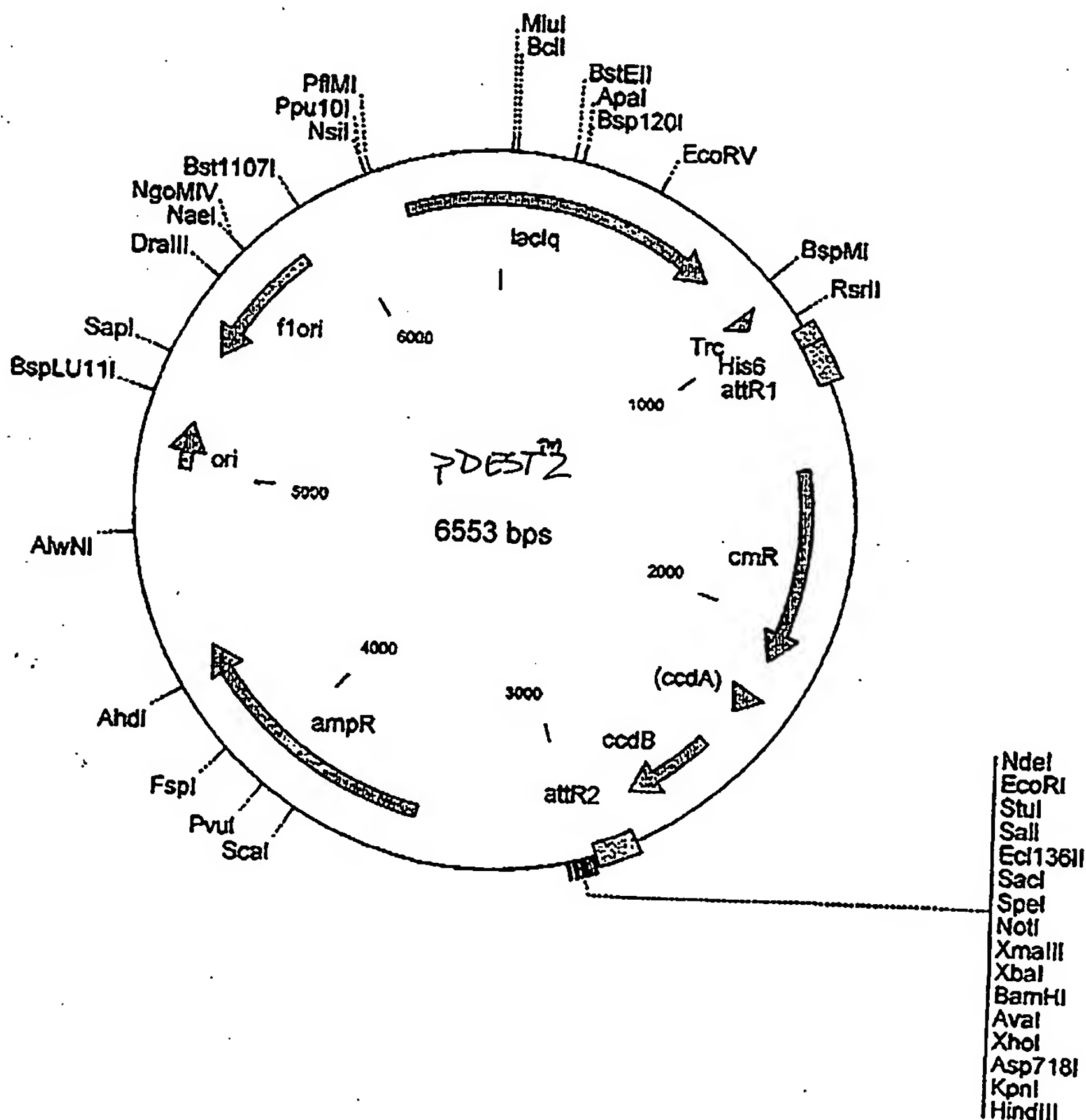
FIGURE 21D

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Figure 22A: pDEST2

His6 fusions in E. coli

970 aat att ctg aaa tga gct <sup>-35</sup> gtt gac aat tad tca tcc ggt ccg <sup>-10</sup> tat aat ctg  
 tta taa gac ttt act cga gaa ctg tca att agt agg cca ggc ata tta gac  
 1021 tgg <sup>RNA</sup> aat tgt gag cgg ata aca att tca cac agg aaa cag acc atg tcg tyr  
 acc tta aca ctc gcc tat tgt taa agt gtg tcc ttt gtc tgg tac agc atg  
 1072 <sup>Tyr His His His His His His</sup> <sup>Glu Ile</sup> <sup>Tyr Ser</sup> <sup>Tyr</sup> <sup>attR1</sup>  
 tac cat cac cat cac cat cac ggt atc aca agt tgg tca aca aca gct gaa  
 atg gta gtg gta gtg gta gtg ccg tag tgt tca aac atg ttt ttt cga cgt





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## pDEST2 6553 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
912..962	Trc
1223..1009	attR1
1473..2132	CmR
2252..2336	inactivated ccdA
2474..2779	ccdB
2820..2944	attR2
3509..4414	ampR
5015..5175	ori
5415..5852	flori (f1 intergenic region)
6225..752	lacIq

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1  GCGCGTGCAC AATCTTCTCG CGCAACGCGT CAGTGGGCTG ATCATTAACT ATCCGCTGGA
61 TGACCAGGAT GCCATTGCTG TGGAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA
121 TGTCTCTGAC CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT
181 GGGCGTGGAG CATCTGGTGC CATTGGGTCA CCAGCAAATC GCGCTGTTAG CGGGCCCAT
241 AAGTTCTGTC TCGGCGCGTC TCGCTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA
301 AATTCAGCCG ATAGCGGAAC GGGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAAC
361 CATGCAAATG CTGAATGAGG GCATCGTTCC CACTGCGATG CTGGTTGCCA ACGATCAGAT
421 GCGCTGGGCG GCAATGCGCG CCATTACCGA GTCCGGGCTG CGCGTTGGTG CGGATATCTC
481 GGTAGTGGGA TACGACGATA CCGAAGACAG CTCATGTTAT ATCCCGCCGT CAACCACCAT
541 CAAACAGGAT TTTCGCCTGC TGGGGCAAAC CAGCGTGGAC CGCTTGCTGC AACTCTCTCA
601 GGGCCAGGCG GTGAAGGGCA ATCAGCTGTT GCGCGTCTCA CTGGTGAAAA GAAAAACCAC
661 CCTGGCACCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT
721 GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT
781 AGCGCGAATT GATCTGGTTT GACAGCTTAT CATCGACTGC ACGGTGCACC AATGCTTCTG
841 GCGTCAGGCA GCCATCGGAA GCTGTGGTAT GGCTGTGCAG GTCGTAAATC ACTGCATAAT
901 TCGTGTGCTC CAAGGCGCAC TCCCGTTCTG GATAATGTTT TTTGCGCCGA CATCATAACG
961 GTTCTGGCAA ATATTCTGAA ATGAGCTGTT GACAATTAAT CATCCGGTCC GTATAATCTG
1021 TGGAATTGTG AGCGGATAAC AATTTACAC AGGAAACAGA CCATGTCGTA CTACCATCAC
1081 CATCACCATC ACGGCATCAC AAGTTTGTAC AAAAAAGCTG AACGAGAAAC GTAAATGAT
1141 ATAAATATCA ATATATTAAT TTAGATTTTG CATAAAAAAC AGACTACATA ATACTGTAAA
1201 ACACAACATA TCCAGTCACT ATGGCGGCCG CTAAGTTGGC AGCATCACCC GACGCACTTT
1261 GCGCCGAATA AATACCTGTG ACGGAAGATC ACTTCGCAGA ATAAATAAAT CCTGGTGTCC
1321 CTGTTGATAC CGGGAAGCCC TGGGCCAACT TTTGGCGAAA ATGAGACGTT GATCGGCACG
1381 TAAGAGGTTT CAACTTTCAC CATAATGAAA TAAGATCACT ACCGGGCGTA TTTTGTGAGT
1441 TATCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA AAAAATCACT GGATATACCA
1501 CCGTTGATAT ATCCCAATGG CATCGTAAAG AACATTTTGA GGCATTTTCA TCAGTTGCTC
1561 AATGTACCTA TAACCAGACC GTTCAGCTGG ATATTACGGC CTTTTTAAAG ACCGTAAAGA
1621 AAAATAAGCA CAAGTTTATAT CCGGCCTTTA TTCACATTCT TGCCCGCCTG ATGAATGCTC
1681 ATCCGGAATT CCGTATGGCA ATGAAAGACG GTGAGCTGGT GATATGGGAT AGTGTTTACC
1741 CTTGTTACAC CGTTTTCCAT GAGCAAACCT AAACGTTTTT ATCGCTCTGG AGTGAATACC
1801 ACGACGATTT CCGGCAGTTT CTACACATAT ATTGCAAGA TGTGGCGTGT TACGGTGAAA
1861 ACCTGGCCTA TTTCCCTAAA GGGTTTATTG AGAATATGTT TTTGCTCTCA GCCAATCCCT
1921 GGGTGAGTTT CACCAAGTTT GATTTAAACG TGGCCAATAT GGACAACCTC TTCGCCCCCG
1981 TTTTACCAT GGGCAAATAT TATACGCAAG GCGACAAGGT GCTGATGCCG CTGGCGATTC
2041 AGGTTTCATCA TGCCGTCTGT GATGGCTTCC ATGTCGGCAG AATGCTTAAT GAATTACAAC
2101 AGTACTGCGA TGAGTGGCAG GGCGGGGCGT AAACGCGTGG ATCCGGCTTA CTAAGAGCCA
2161 GATAACAGTA TGCGTATTTG CGCGCTGATT TTTGCGGTAT AAGAATATAT ACTGATATGT
2221 ATACCCGAAG TATGTCAAAA AGAGGTGTGC TATGAAGCAG CGTATTACAG TGACAGTTGA
2281 CAGCGACAGC TATCAGTTGC TCAAGGCATA TATGATGTCA ATATCTCCGG TCTGGTAAGC
2341 ACAACCATGC AGAATGAAGC CCGTCGTCTG CGTGCCGAAC GCTGGAAAGC GGAAATCAG
2401 GAAGGGATGG CTGAGGTCCG CCGGTTTATT GAAATGAACG GCTCTTTTGC TGACGAGAAC
2461 AGGGACTGGT GAAATGCAGT TTAAGTTTAA CACCTATAAA AGAGAGAGCC GTTATCGTCT
2521 GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG CGACGGATGG TGATCCCCCT-

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FIGURE 22B



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2581 GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA CTTTACCCGG TGGTG CATAT  
2641 CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC AGTGTGCCGG TCTCCGTTAT  
2701 CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC ATCAAAAACG CCATTAACCT  
2761 GATGTTCTGG GGAATATAAA TGTCAGGCTC CTTTATACAC AGCCAGTCTG CAGGTCGACC  
2821 ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAGT CTGTTTTTTA TGCAAAATCT  
2881 AATTTAATAT ATTGATATTT ATATCATTTT ACGTTTCTCG TTCAGCTTTC TTGTACAAAG  
2941 TGGTGATGCC CATATGGGAA TTCAAAGGCC TACGTCGACG AGCTCACTAG TCGCGGCCGC  
3001 TTCTAGAGGA TCCCTCGAGG CATGCGGTAC CAAGCTTGGC TGTTTTGGCG GATGAGAGAA  
3061 GATTTTCAGC CTGATACAGA TTAAATCAGA ACGCAGAAGC GGTCTGATAA AACAGAATTT  
3121 GCCTGGCGGC AGTAGCGCGG TGGTCCCACC TGACCCCATG CCGAACTCAG AAGTGAAACG  
3181 CCGTAGCGCC GATGGTAGTG TGGGGTCTCC CCATGCGAGA GTAGGGAAC TCCAGGCATC  
3241 AAATAAACG AAAGGCTCAG TCGAAAGACT GGGCCTTTTCG TTTTATCTGT TGTTTGTCCG  
3301 TGAACGCTCT CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT GCGAAGCAAC  
3361 GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC CAGGCATCAA ATTAAGCAGA  
3421 AGGCCATCCT GACGGATGGC CTTTTGCGT TTCTACAAAC TCTTTTTGTT TATTTTTCTA  
3481 AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA  
3541 TTGAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC  
3601 GGCATTTTGC CTTCTGTTT TTGCTCACC AGAAACGCTG GTGAAAGTAA AAGATGCTGA  
3661 AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT  
3721 TGAGAGTTTT CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG  
3781 TGGCGCGGTA TTATCCCGTG TTGACGCCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA  
3841 TTCTCAGAA GACTTGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT  
3901 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT  
3961 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT TTTTGCACA ACATGGGGGA  
4021 TCATGTAAC CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA  
4081 GCGTGACACC ACGATGCCTA CAGCAATGGC AACAACGTTG CGCAAACAT TAACTGGCGA  
4141 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC  
4201 AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA AATCTGGAGC  
4261 CCGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG  
4321 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT  
4381 CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA  
4441 TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT  
4501 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA  
4561 CCCCCTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG  
4621 CTTGCAAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC  
4681 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT  
4741 AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACCTCGC  
4801 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT  
4861 GGA CTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTGCG GGCTGAACGG GGGGTTCTGT  
4921 CACACAGCCC AGCTTGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT  
4981 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG  
5041 GGTCCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCTGGT ATCTTTATAG  
5101 TCCTGTCCGG TTTCCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG  
5161 GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG  
5221 GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC  
5281 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT  
5341 GAGCGAGGAA GCGGAAGAGC GCCTGATGCG GTATTTTCTC CTTACGCATC TGTGCGGTAT  
5401 TTCACACCGC ATAATTTTGT TAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT  
5461 TAACCAATAG GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA CCGAGATAGG  
5521 GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA AAGAACGTGG ACTCCAACGT  
5581 CAAAGGGCGA AAAACCGTCT ATCAGGGCGA TGGCCCACTA CGTGAACCAT CACCCTAATC  
5641 AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC ACTAAATCGG AACCTAAAG GGAGCCCCCG  
5701 ATTTAGAGCT TGACGGGGAA AGCCGGCGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA  
5761 AGGAGCGGGC GCTAGGGCGC TGGCAAGTGT AGCGGTCACG CTGCGCGTAA CCACCACACC  
5821 CGCCGCGCTT AATGCGCCGC TACAGGGCGC GTCCCATTCG CCATTCAGGC TGCTATGGTG  
5881 CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAGTATACAC TCCGCTATCG  
5941 CTACGTGACT GGGTCATGGC TGCGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA  
6001 CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC CGGGAGCTGC

FIGURE 22C

6061 ATGTGTCAGA GGTTCACCG GTCATCACCG AAACGCGCGA GGCAGCAGAT CAATTCGCGC  
6121 GCGAAGGCGA AGCGGCATGC ATTTACGTTG ACACCATCGA ATGGTGCAAA ACCTTTCGCG  
6181 GTATGGCATG ATAGCGCCCG GAAGAGAGTC AATTCAGGGT GGTGAATGTG AAACCAGTAA  
6241 CGTTATACGA TGTCGCAGAG TATGCCGGTG TCTCTTATCA GACCGTTTCC CGCGTGGTGA  
6301 ACCAGGCCAG CCACGTTTCT GCGAAAACGC GGGAAAAAGT GGAAGCGGCG ATGGCGGAGC  
6361 TGAATTACAT TCCAACCGC GTGGCACAAC AACTGGCGGG CAAACAGTCG TTGCTGATTG  
6421 GCGTTGCCAC CTCCAGTCTG GCCCTGCACG CGCCGTCGCA AATTGTCGCG GCGATTAAAT  
6481 CTCGCGCCGA TCAACTGGGT GCCAGCGTGG TGGTGTGAT GGTAGAACGA AGCGGCGTCG  
6541 AAGCCTGTAA AGC

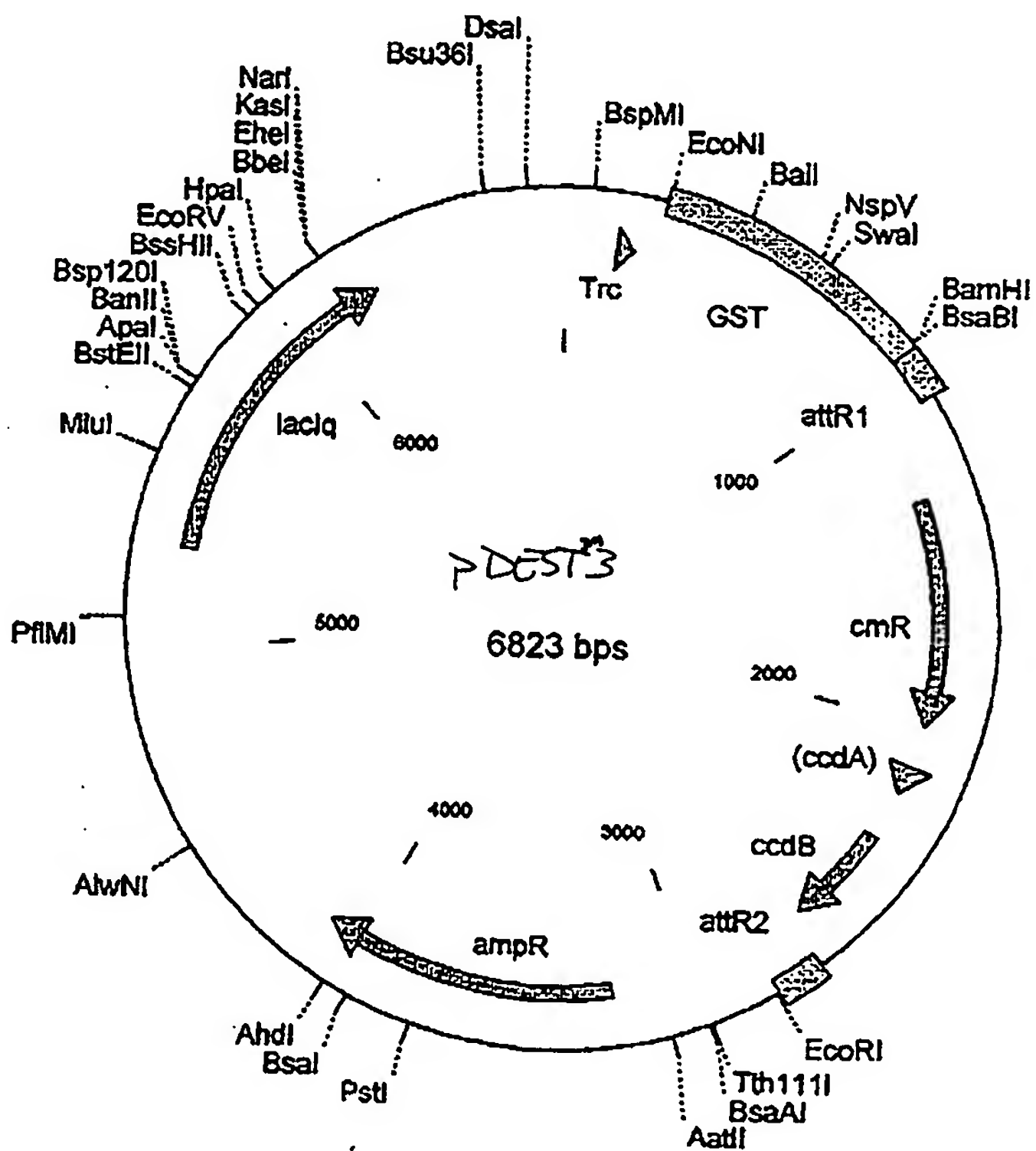
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Figure 23A: pDEST3

## GST fusions in E. coli

154 cgg ttc tgg caa ata ttc tga aat gag ctg <sup>-35</sup> ctg aca att <sup>Trc promoter</sup> aat cat cgg ctc  
 gcc aag acc gtt tat aag act tta ctc gac aac tgt taa tta gta gcc gag  
 205 <sup>-10</sup> gta taa <sup>→ mRNA</sup> ggt gtg gaa ttg tga gcg gat aac aat ttc aca cag gaa aca gta  
 cat att aca cac ctt aac act cgc cta ttg tta aag tgt gtc ctt tgt cat  
 256 <sup>M S P I L</sup> <sup>→ GST</sup> ttc atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc  
 aag tac agg gga tat gat cca ata acc ttt taa ttc ccg gaa cac gtt ggg

919 " GST → R G S R R A S V G S P S T S  
 ctg gtt ccg cgt gga tct cgt cgt gca tct gtt gga tcc cca tca aca agt  
 gac caa ggc gca cct aga gca gca cgt aga caa cct agg ggt agt tgt tca  
 970 <sup>H Y K K</sup> ~~tgg xac aac aac gct gaa cga gaa acg taa aat gat ata aat aat aat aat ata~~  
~~aac atg tct ttt cga cct gct cct tgc att tta cta tat tta tag tta tat~~



## pDEST3 6823 bp

Location (Base Nos.)	Gene Encoded
150..200	Trc
1087..963	attR1
1337..1996	CmR
2116..2200	inactivated ccdA
2338..2643	ccdB
2684..2808	attR2
3231..4091	ampR
5295..6254	lacIq

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1 ACGTTATCGA CTGCACGGTG CACCAATGCT TCTGGCGTCA GGCAGCCATC GGAAGCTGTG
61 GTATGGCTGT GCAGGTCGTA AATCACTGCA TAATTCGTGT CGCTCAAGGC GCACTCCCGT
121 TCTGGATAAT GTTTTTTTCG CCGACATCAT AACGGTTCTG GCAAATATTC TGAAATGAGC
181 TGTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAATTTCA
241 CACAGGAAAC AGTATTCATG TCCCCTATAC TAGGTTATTG GAAAATTAAG GGCCTTGTGC
301 AACCCACTCG ACTTCTTTTG GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC
361 GCGATGAAGG TGATAAATGG CGAAACAAAA AGTTTGAATT GGGTTTGGAG TTTCCCAATC
421 TTCCTTATTA TATTGATGGT GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA
481 TAGCTGACAA GCACAACATG TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC
541 TTGAAGGAGC GGTTTTGGAT ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT
601 TTGAAACTCT CAAAGTTGAT TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTTGAAG
661 ATCGTTTATG TCATAAAACA TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT
721 TGTATGACGC TCTTGATGTT GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCCAA
781 AATTAGTTTG TTTTAAAAAA CGTATTGAAG CTATCCCACA AATTGATAAG TACTTGAAAT
841 CCAGCAAGTA TATAGCATGG CCTTTCAGAG GCTGGCAAGC CACGTTTGGT GGTGGCGACC
901 ATCCTCCAAA ATCGGATCTG GTTCCGCGTG GATCTCGTCG TGCATCTGTT GGATCCCCAT
961 CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA TGATATAAAT ATCAATATAT
1021 TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG TAAACACAA CATATCCAGT
1081 CACTATGGCG GCCGCTAAGT TGGCAGCATC ACCCGACGCA CTTTGCGCCG AATAAATACC
1141 TGTGACGGAA GATCACTTCG CAGAATAAAT AAATCCTGGT GTCCCTGTTG ATACCGGGAA
1201 GCCCTGGGCC AACTTTTGGC GAAAATGAGA CGTTGATCGG CACGTAAGAG GTTCCAACCT
1261 TCACCATAAT GAAATAAGAT CACTACCGGG CGTATTTTTT GAGTTATCGA GATTTTCAGG
1321 AGCTAAGGAA GCTAAAATGG AGAAAAAAT CACTGGATAT ACCACCGTTG ATATATCCCA
1381 ATGGCATCGT AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA
1441 GACCGTTCAG CTGGATATTA CGGCCTTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT
1501 TTATCCGGCC TTTATTCACA TTCTTGCCCG CTTGATGAAT GCTCATCCGG AATTCCGTAT
1561 GGCAATGAAA GACGGTGAGC TGGTGATATG GGATAGTGTT CACCCTTGTT ACACCGTTTT
1621 CCATGAGCAA ACTGAAACGT TTTCATCGCT CTGGAGTGAA TACCACGACG ATTTCCGGCA
1681 GTTCTACAC ATATATTTCG AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC
1741 TAAAGGGTTT ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCAG
1801 TTTTGATTTA AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA CCATGGGCAA
1861 ATATTATACG CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTTT ATCATGCCGT
1921 CTGTGATGGC TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT GCGATGAGTG
1981 GCAGGGCGGG GCGTAAAGAT CTGGATCCGG CTTACTAAAA GCCAGATAAC AGTATGCGTA
2041 TTTGCGCGCT GATTTTTTCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC
2101 AAAAAGAGGT GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA CAGCTATCAG
2161 TTGCTCAAGG CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC ATGCAGAATG
2221 AAGCCCCTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG
2281 TCGCCCGGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC TGGTGAAATG
2341 CAGTTTAAGG TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT GGATGTACAG
2401 AGTGATATTA TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG TGCACGCTGT
2461 CTGTCAGATA AAGTCTCCCG TGAACTTTAC CCGGTGGTGC ATATCGGGGA TGAAAGCTGG
2521 CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGGCT
2581 GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGGAATA
2641 TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG ACTGGATATG
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2701 TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA ATATATTGAT  
2761 ATTTATATCA TTTTACGTTT CTCGTTGAGC TTTCTTGAC AAAGTGGTTG ATGGGAATTC  
2821 ATCGTGACTG ACTGACGATC TGCCTCGCGC GTTTCGGTGA TGACGGTGAA AACCTCTGAC  
2881 ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG AGCAGACAAG  
2941 CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CGCAGCCATG ACCCAGTCAC  
3001 GTAGCGATAG CGGAGTGTAT AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT  
3061 TATAGGTTAA TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA  
3121 ATGTGCGCGG AACCCTTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA  
3181 TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC  
3241 AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCTTCCT GTTTTTGCTC  
3301 ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGTT  
3361 ACATCGAAGT GGATCTCAAC AGCGGTAAAG TCCTTGAGAG TTTTCGCCCC GAAGAACGTT  
3421 TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTTGACG  
3481 CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT  
3541 CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG  
3601 CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA  
3661 AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG  
3721 AACC GGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA  
3781 TGGCAACAAC GTTGC GCAAA CTATTAAGT GCGAACTACT TACTCTAGCT TCCCGGCAAC  
3841 AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC  
3901 CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA  
3961 TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA  
4021 GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA  
4081 AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACCTC  
4141 ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC  
4201 CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT  
4261 CTTGAGATCC TTTTTCCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC  
4321 CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT  
4381 TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT  
4441 TCAAGAACTC TGTAGCACC GCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG  
4501 CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG TTACCGGATA  
4561 AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACCA GCCCAGCTTG GAGCGAACGA  
4621 CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG  
4681 GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCAGGAGGG  
4741 AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CCGGTTTCGC CACCTCTGAC  
4801 TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA  
4861 ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG  
4921 CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC  
4981 GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA  
5041 TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATAAAT TCCGACACCA  
5101 TCGAATGGTG CAAAACCTTT CGCGGTATGG CATGATAGCG CCCGGAAGAG AGTCAATTCA  
5161 GGGTGGTGAA TGTGAAACCA GTAACGTTAT ACGATGTCGC AGAGTATGCC GGTGTCTCTT  
5221 ATCAGACCGT TTCCCGCGTG GTGAACCAGG CCAGCCACGT TTCTGCGAAA ACGCGGGAAA  
5281 AAGTGGAAGC GGCGATGGCG GAGCTGAATT ACATTCCCAA CCGCGTGGCA CAACAACTGG  
5341 CGGGCAAACA GTCGTTGCTG ATTGGCGTTG CCACCTCCAG TCTGGCCCTG CACGCGCCGT  
5401 CGCAAATTGT CGCGGCGATT AAATCTCGCG CCGATCAACT GGGTGCCAGC GTGGTGGTGT  
5461 CGATGGTAGA ACGAAGCGGC GTCGAAGCCT GTAAAGCGGC GGTGCACAAT CTTCTCGCGC  
5521 AACGCGTCAG TGGGCTGATC ATTAAGTATC CGCTGGATGA CCAGGATGCC ATTGCTGTGG  
5581 AAGCTGCCTG CACTAATGTT CCGGCGTTAT TTCTTGATGT CTCTGACCAG ACACCCATCA  
5641 ACAGTATTAT TTTCTCCCAT GAAGACGGTA CGCGACTGGG CGTGGAGCAT CTGGTCGCAT  
5701 TGGGTCACCA GCAAATCGCG CTGTTAGCGG GCCCATTAAG TTCTGTCTCG GCGCGTCTGC  
5761 GTCTGGCTGG CTGGCATAAA TATCTCACTC GCAATCAAAT TCAGCCGATA GCGGAACGGG  
5821 AAGGCGACTG GAGTGCCATG TCCGGTTTTT AACAAACCAT GCAAATGCTG AATGAGGGCA  
5881 TCGTTCCAC TGCGATGCTG GTTGCCAACG ATCAGATGGC GCTGGGCGCA ATGCGCGCCA  
5941 TTACCGAGTC CGGGCTGCGC GTTGGTGCGG ATATCTCGGT AGTGGGATAC GACGATACCG  
6001 AAGACAGCTC ATGTTATATC CCGCCGTTAA CCACCATCAA ACAGGATTTT CGCCTGCTGG  
6061 GGCAAACCAG CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG AAGGGCAATC  
6121 AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGCGCCCAAT ACGCAAACCG-

Figure 23C

6181 CCTCTCCCCG CGCGTTGGCC GATTCATTAA TGCAGCTGGC ACGACAGGTT TCCCGACTGG  
6241 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTAGC TCACTCATTG GGCACCCAG  
6301 GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT  
6361 CACACAGGAA ACAGCTATGA CCATGATTAC GGATTCACTG GCCGTCGTTT TACAACGTCG  
6421 TGA CTGGGAA AACCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTTCG  
6481 CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TCGCAGCCT  
6541 GAATGGCGAA TGGCGCTTTG CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT  
6601 GGAGTGCGAT CTTCTGAGG CCGATACTGT CGTCGTCCCC TCAAACCTGGC AGATGCACGG  
6661 TTACGATGCG CCCATCTACA CCAACGTAAC CTATCCCATT ACGGTCAATC CGCCGTTTGT  
6721 TCCCACGGAG AATCCGACGG GTTGTTACTC GCTCACATTT AATGTTGATG AAAGCTGGCT  
6781 ACAGGAAGGC CAGACGCGAA TTATTTTGA TGGCGTTGGA ATT





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## pDEST4 6964 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
964..1003	Trc
1577..1453	attR1
1827..2486	CmR
2606..2690	inactivated ccdA
2828..3133	ccdB
3174..3298	attR2
3872..4777	ampR
5378..5538	ori
5778..6215	flori (f1 intergenic region)
6587..704	lacIq

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1 CTATCCGCTG GATGACCAGG ATGCCATTGC TGTGGAAGCT GCCTGCACTA ATGTTCCGGC
61 GTTATTTCTT GATGTCTCTG ACCAGACACC CATCAACAGT ATTATTTTCT CCCATGAAGA
121 CGGTACGCGA CTGGGCGTGG AGCATCTGGT CGCATTGGGT CACCAGCAA TCGCGCTGTT
181 AGCGGGCCCA TTAAGTTCTG TCTCGGCGCG TCTGCGTCTG GCTGGCTGGC ATAAATATCT
241 CACTCGCAAT CAAATTCAGC CGATAGCGGA ACGGGAAGGC GACTGGAGTG CCATGTCCGG
301 TTTTCAACAA ACCATGCAA TGCTGAATGA GGGCATCGTT CCCACTGCGA TGCTGGTTGC
361 CAACGATCAG ATGGCGCTGG GCGCAATGCG CGCCATTACC GAGTCCGGGC TCGCGCTTGG
421 TCGGATATC TCGGTAGTGG GATACGACGA TACCGAAGAC AGCTCATGTT ATATCCCGCC
481 GTCAACCACC ATCAAACAGG ATTTTCGCCT GCTGGGGCAA ACCAGCGTGG ACCGCTTGCT
541 GCAACTCTCT CAGGGCCAGG CCGTGAAGGG CAATCAGCTG TTGCCCGTCT CACTGGTGAA
601 AAGAAAAACC ACCCTGGCAC CCAATACGCA AACCCTCTCT CCCCCTGCGT TGGCCGATTG
661 ATTAATGCAG CTGGCACGAC AGGTTTCCCG ACTGGAAGGC GGGCAGTGAG CGCAACGCAA
721 TTAATGTGAG TTAGCGCGAA TTGATCTGGT TTGACAGCTT ATCATCGACT GCACGGTGCA
781 CCAATGCTTC TGGCGTCAGG CAGCCATCGG AAGCTGTGGT ATGGCTGTGC AGGTCGTAAA
841 TCACTGCATA ATTCGTGTCT CTCAAGGCGC ACTCCCGTTC TGGATAATGT TTTTGTGCGC
901 GACATCATAA CGGTTCTGGC AAATATTCTG AAATGAGCTG TTGACAATTA ATCATCCGGT
961 CCGTATAATC TGTGGAATTG TGAGCGGATA ACAATTTTAC ACAGGAAACA GACCATGGGT
1021 CATCATCATC ATCATCACGA TTACGATATC CCAACGACCG AAAACCTGTA TTTTCAGGGC
1081 GCCCATATGA GCGATAAAAT TATTCACCTG ACTGACGACA GTTTTGACAC GGATGTACTC
1141 AAAGCGGACG GGGCGATCCT CGTCGATTTT TGGGCAGAGT GGTGCGGTCC GTGCAAAATG
1201 ATCGCCCCGA TTCTGGATGA AATCGCTGAC GAATATCAGG GCAAACGAC CGTTGCAAAA
1261 CTGAACATCG ATCAAAACCC TGGCACTGCG CCGAAATATG GCATCCGTGG TATCCCGACT
1321 CTGCTGCTGT TCAAAAACGG TGAAGTGGCG GCAACCAAAG TGGGTGCACT GTCTAAAGGT
1381 CAGTTGAAAG AGTTCCTCGA CGCTAACCTG GCCGGTCTG GTTCTGGTGA TGACGATGAC
1441 AAGGTACCCA TCACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA TGATATAAAT
1501 ATCAATATAT TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG TAAACACAA
1561 CATATCCAGT CACTATGGCG GCCGCTAAGT TGGCAGCATC ACCCGACGCA CTTTGCGCCG
1621 AATAAATACC TGTGACGGAA GATCACTTCG CAGAATAAAT AAATCCTGGT GTCCCTGTTG
1681 ATACCGGGAA GCCCTGGGCC AACTTTTGGC GAAATGAGA CGTTGATCGG CACGTAAGAG
1741 GTTCCAACCT TCACCATAAT GAAATAAGAT CACTACCGGG CGTATTTTTT GAGTTATCGA
1801 GATTTTCAGG AGCTAAGGAA GCTAAATGAG AGAAAAAAT CACTGGATAT ACCACCGTTG
1861 ATATATCCCA ATGGCATCGT AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA
1921 CCTATAACCA GACCGTTCAG CTGGATATTA CGGCCTTTTT AAAGACCGTA AAGAAAAATA
1981 AGCACAAGTT TTATCCGGCC TTTATTCACA TTCTTGCCCC CCTGATGAAT GCTCATCCGG
2041 AATTCCGTAT GGCAATGAAA GACGGTGAGC TGGTGATATG GGATAGTGTT CACCCTTGTT
2101 ACACCGTTTT CCATGAGCAA ACTGAAACGT TTTCATCGCT CTGGAGTGAA TACCACGACG
2161 ATTTCCGGCA GTTTCTACAC ATATATTCGC AAGATGTGGC GTGTTACGGT GAAAACCTGG
2221 CCTATTTCCC TAAAGGGTTT ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA
2281 GTTTCACCAG TTTTGATTTA AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA
2341 CCATGGGCAA ATATTATACG CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTTT
2401 ATCATGCCGT CTGTGATGGC TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT
2461 GCGATGAGTG GCAGGGCGGG GCGTAAACGC GTGGATCCGG CTTACTAAAA GCCAGATAAC
2521 AGTATGCGTA TTTGCGCGCT GATTTTTCGC GTATAAGAAT ATATACTGAT ATGTATACCC-

```

FIGURE 24B

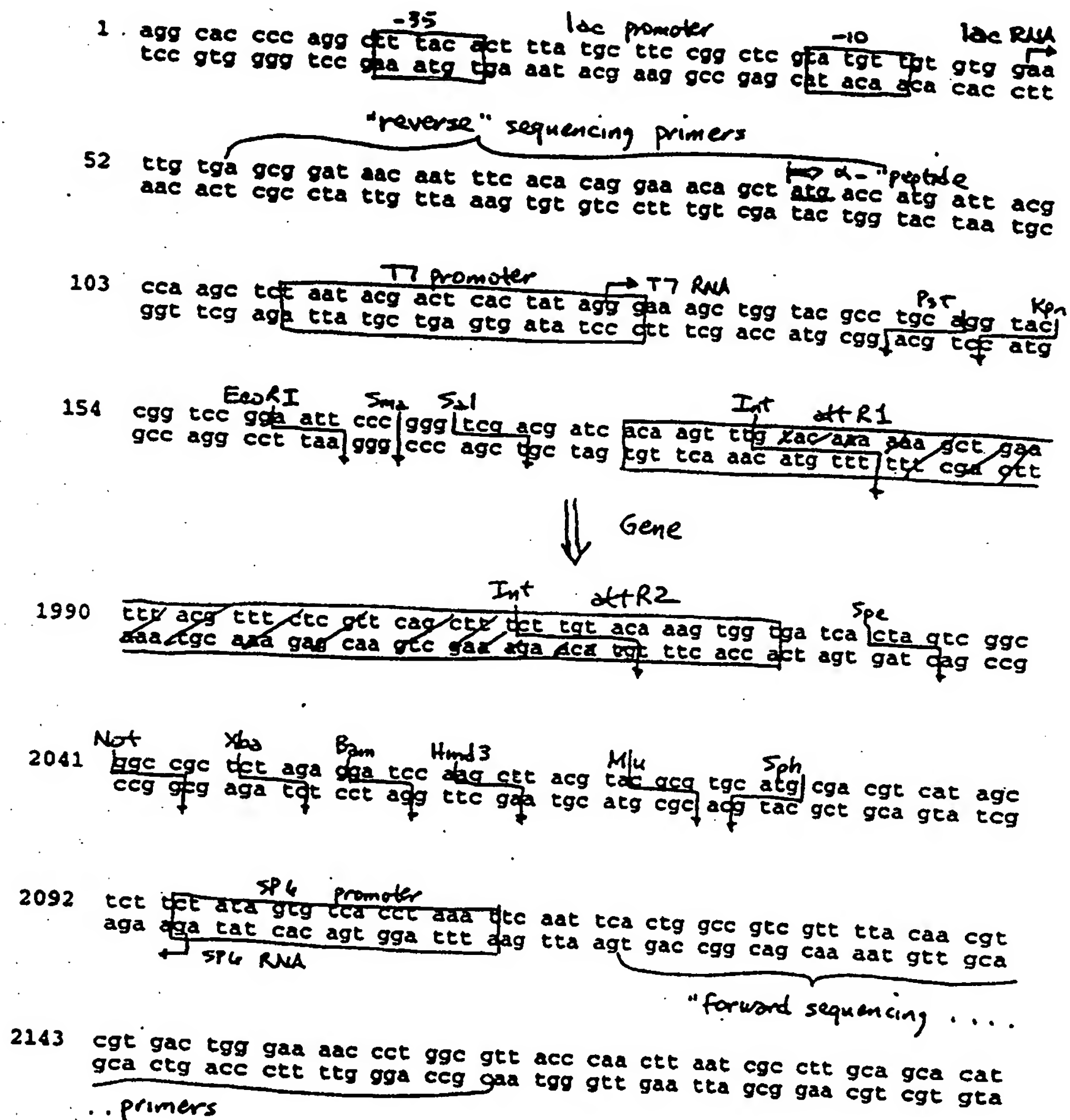
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2641 CAGCTATCAG TTGCTCAAGG CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC  
2701 ATGCAGAATG AAGCCCGTCG TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG  
2761 ATGGCTGAGG TCGCCCGGTT TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC  
2821 TGGTGAAATG CAGTTTAAAG TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT  
2881 GGATGTACAG AGTGATATTA TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG  
2941 TGCACGTCTG CTGTCAGATA AAGTCTCCCG TGAACTTTAC CCGGTGGTGC ATATCGGGGA  
3001 TGAAAGCTGG CGCATGATGA CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA  
3061 AGAAGTGGCT GATCTCAGCC ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT  
3121 CTGGGGAATA TAAATGTCAG GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG  
3181 ACTGGATATG TTGTGTTTTA CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA  
3241 ATATATTGAT ATTTATATCA TTTTACGTTT CTCGTTTCAGC TTTCTTGAC AAAGTGGTGA  
3301 TGGGGATCCT CTAGAGTCGA CCTGCAGTAA TCGTACAGGG TAGTACAAAT AAAAAAGGCA  
3361 CGTCAGATGA CGTGCCTTTT TTCTTGAGAG CAGTAAGCTT GGCTGTTTTG GCGGATGAGA  
3421 GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA TAAAACAGAA  
3481 TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAAC CAGAAGTGAA  
3541 ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA ACTGCCAGGC  
3601 ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCCT TCGTTTTATC TGTTGTTTGT  
3661 CGGTGAACGC TCTCCTGAGT AGGACAAATC CGCCGGGAGC GGATTTGAAC GTTGCGAAGC  
3721 AACGGCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC TGCCAGGCAT CAAATTAAGC  
3781 AGAAGGCCAT CCTGACGGAT GGCCTTTTTG CGTTTCTACA AACTCTTTTT GTTTATTTTT  
3841 CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA  
3901 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT  
3961 TGCGGCATTT TGCTTCCCTG TTTTGTCTCA CCCAGAAACG CTGGTGAAAG TAAAAGATGC  
4021 TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT  
4081 CCTTGAGAGT TTTGCCCCG AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT  
4141 ATGTGGCGCG GTATTATCCC GTGTTGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA  
4201 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG  
4261 CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG AGTGATAACA CTGCGGCCAA  
4321 CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG  
4381 GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA  
4441 CGAGCGTGAC ACCACGATGC CTACAGCAAT GGCAACAACG TTGCGCAAAC TATTAAGTGG  
4501 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT  
4561 TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG TTTATTGCTG ATAAATCTGG  
4621 AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC  
4681 CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA  
4741 GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC  
4801 ATATATACTT TAGATTGATT TAAAACCTCA TTTTAAATTT AAAAGGATCT AGGTGAAGAT  
4861 CCTTTTGTAT AATCTCATGA CCAAATCCC TTAACGTGAG TTTTCGTTCC ACTGAGCGTC  
4921 AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG  
4981 CTGCTTGCAA AAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT  
5041 ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT  
5101 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACC GC CTACATACCT  
5161 CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT GTCTTACCGG  
5221 GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTT  
5281 GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA  
5341 GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGCGG GACAGGTATC CGGTAAGCGG  
5401 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA  
5461 TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT GCTCGTCAGG  
5521 GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG  
5581 CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT  
5641 TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC  
5701 AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG  
5761 TATTTACAC CGCATAATTT TGTTAAATTT CGCGTTAAAT TTTTGTAAA TCAGCTCATT  
5821 TTTTAACCAA TAGGCCGAAA TCGGCAAAAT CCCTTATAAA TCAAAGAAT AGACCGAGAT  
5881 AGGGTTGAGT GTTGTTCAG TTTGGAACAA GAGTCCACTA TTAAAGAACC TGGACTCCAA  
5941 CGTCAAAGGG CGAAAAACCG TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCCTA  
6001 ATCAAGTTTT TTGGGGTCGA GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC-

6061 CCGATTTAGA GCTTGACGGG GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC  
6121 GAAAGGAGCG GCGCTAGGG CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC  
6181 ACCCGCCGCG CTTAATGCGC CGCTACAGGG CGCGTCCATT CGCCATTCAG GCTGCTATGG  
6241 TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAGTATAC ACTCCGCTAT  
6301 CGCTACGTGA CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT  
6361 GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCCGGGAGCT  
6421 GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGCAG ATCAATTTCG  
6481 GCGCGAAGGC GAAGCGGCAT GCATTTACGT TGACACCATC GAATGGTGCA AAACCTTTCG  
6541 CGGTATGGCA TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACCACT  
6601 AACGTTATAC GATGTCGCAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT  
6661 GAACCAGGCC AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA  
6721 GCTGAATTAC ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT  
6781 TGGCGTTGCC ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCG CGGCGATTAA  
6841 ATCTCGCGCC GATCAACTGG GTGCCAGCGT GGTGGTGTCT ATGGTAGAAC GAAGCGGCGT  
6901 CGAAGCCTGT AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGCGTCAGTN GGGCTGATCA  
6961 TTAA

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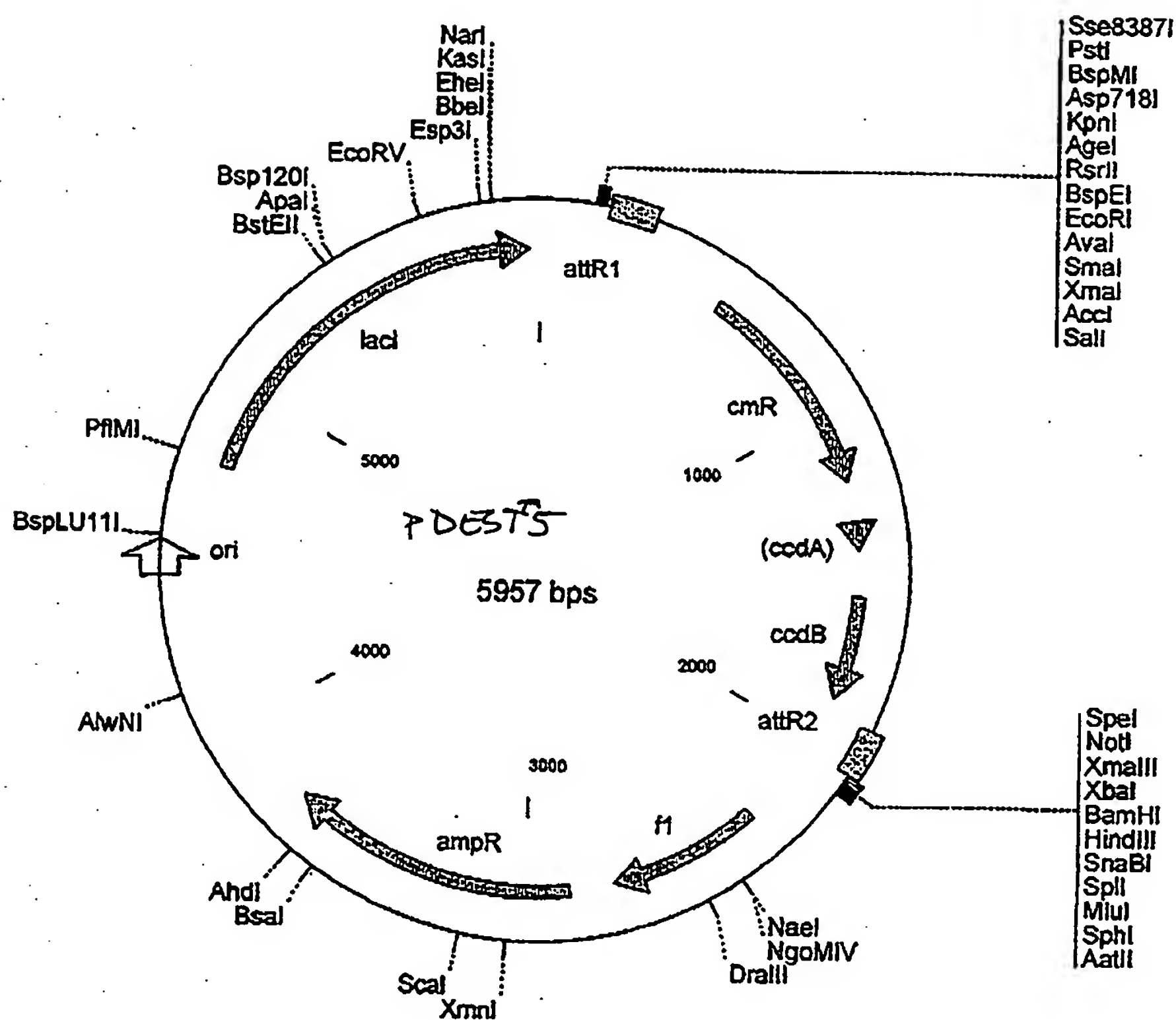
Figure 25A

pDEST5

pSPORT '+' (for sequencing, probes,  
phagemid)

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Figure 25B 7DEST5 (cont'd)





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## pDEST5 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
305..181		attR1
555..1214		CmR
1334..1418		inactivated ccdA
1556..1861		ccdB
1902..2026		attR2
2278..2733		f1 (f1 intergenic region)
2865..3722		ampR
5378..5538		ori
4756..5922		lacI

1	AGGCACCCCA	GGCTTTACAC	TTTATGCTTC	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG
61	GATAACAATT	TCACACAGGA	AACAGCTATG	ACCATGATTA	CGCCAAGCTC	TAATACGACT
121	CACTATAGGG	AAAGCTGGTA	CGCCTGCAGG	TACCGGTCCG	GAATTCCTCG	GTCGACGATC
181	ACAAGTTTGT	ACAAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA
241	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA
301	CTATGGCGGC	CGCTAAGTTG	GCAGCATCAC	CCGACGCACT	TTGCGCCGAA	TAAATACCTG
361	TGACGGAAGA	TCACTTCGCA	GAATAAATAA	ATCCTGGTGT	CCCTGTTGAT	ACCGGGAAGC
421	CCTGGGCCAA	CTTTTGCGCA	AAATGAGACG	TTGATCGGCA	CGTAAGAGGT	TCCAACCTTC
481	ACCATAATGA	AATAAGATCA	CTACCGGGCG	TATTTTTTGA	GTTATCGAGA	TTTTTCAGGAG
541	CTAAGGAAGC	TAAAATGGAG	AAAAAATCA	CTGGATATAC	CACCGTTGAT	ATATCCCAAT
601	GGCATCGTAA	AGAACATTTT	GAGGCATTTT	AGTCAGTTGC	TCAATGTACC	TATAACCAGA
661	CCGTTTCAGCT	GGATATTACG	GCCTTTTTAA	AGACCGTAAA	GAAAAATAAG	CACAAGTTTT
721	ATCCGGCCTT	TATTCACATT	CTTGCCCGCC	TGATGAATGC	TCATCCGGAA	TTCCGTATGG
781	CAATGAAAGA	CGGTGAGCTG	GTGATATGGG	ATAGTGTTC	CCCTTGTTAC	ACCGTTTTCC
841	ATGAGCAAAC	TGAAACGTTT	TCATCGCTCT	GGAGTGAATA	CCACGACGAT	TTCCGGCAGT
901	TTCTACACAT	ATATTCGCAA	GATGTGGCGT	GTTACGGTGA	AAACCTGGCC	TATTTCCCTA
961	AAGGGTTTAT	TGAGAATATG	TTTTTCGTCT	CAGCCAATCC	CTGGGTGAGT	TTCACCAGTT
1021	TTGATTTAAA	CGTGGCCAAT	ATGGACAAC	TCTTCGCCCC	CGTTTTCCAC	ATGGGCAAAT
1081	ATTATACGCA	AGGCGACAAG	GTGCTGATGC	CGCTGGCGAT	TCAGGTTTCAT	CATGCCGTCT
1141	GTGATGGCTT	CCATGTCGGC	AGAATGCTTA	ATGAATTACA	ACAGTACTGC	GATGAGTGGC
1201	AGGGCGGGGC	GTAAACGCGT	GGATCCGGCT	TACTAAAAGC	CAGATAACAG	TATGCGTATT
1261	TGCGCGCTGA	TTTTTGCGGT	ATAAGAATAT	ATACTGATAT	GTATACCCGA	AGTATGTCAA
1321	AAAGAGGTGT	GCTATGAAGC	AGCGTATTAC	AGTGACAGTT	GACAGCGACA	GCTATCAGTT
1381	GCTCAAGGCA	TATATGATGT	CAATATCTCC	GGTCTGGTAA	GCACAACCAT	GCAGAATGAA
1441	GCCCGTCGTC	TGCGTGCCGA	ACGCTGGAAA	GCGGAAAATC	AGGAAGGGAT	GGCTGAGGTC
1501	GCCCGGTTTA	TTGAAATGAA	CGGCTCTTTT	GCTGACGAGA	ACAGGGACTG	GTGAAATGCA
1561	GTTTAAGGTT	TACACCTATA	AAAGAGAGAG	CCGTTATCGT	CTGTTTGTGG	ATGTACAGAG
1621	TGATATTATT	GACACGCCCC	GGCGACGGAT	GGTGATCCCC	CTGGCCAGTG	CACGTCTGCT
1681	GTCAGATAAA	GTCTCCCGTG	AACTTTACCC	GGTGGTGCAT	ATCGGGGATG	AAAGCTGGCG
1741	CATGATGACC	ACCGATATGG	CCAGTGTGCC	GGTCTCCGTT	ATCGGGGAAG	AAGTGGCTGA
1801	TCTCAGCCAC	CGCGAAAATG	ACATCAAAAA	CGCCATTAA	CTGATGTTCT	GGGGAATATA
1861	AATGTCAGGC	TCCCTTATAC	ACAGCCAGTC	TGCAGGTCGA	CCATAGTGAC	TGGATATGTT
1921	GTGTTTTACA	GTATTATGTA	GTCTGTTTTT	TATGCAAAAT	CTAATTTAAT	ATATTGATAT
1981	TTATATCATT	TTACGTTTCT	CGTTCAGCTT	TCTTGTAACA	AGTGGTGATC	ACTAGTCGGC
2041	GGCCGCTCTA	GAGGATCCAA	GCTTACGTAC	GCGTGCAATG	GACGTCATAG	CTCTTCTATA
2101	GTGTCACCTA	AATTCAATTC	ACTGGCCGTC	GTTTTACAAC	GTCGTGACTG	GGAAAACCTT
2161	GGCGTTACCC	AACTTAATCG	CCTTGCAGCA	CATCCCCCTT	TCGCCAGCTG	GCGTAATAGC
2221	GAAGAGGCCC	GCACCGATCG	CCCTTCCCAA	CAGTTGCGCA	GCCTGAATGG	CGAATGGACG
2281	CGCCCTGTAG	CGGCGCATTA	AGCGCGGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA
2341	CACTTGCCAG	CGCCCTAGCG	CCCGCTCCTT	TGCTTTTCTT	CCCTTCTTTT	CTCGCCACGT
2401	TCGCCGGCTT	TCCCGTCAA	GCTCTAAATC	GGGGGCTCCC	TTTAGGGTTC	CGATTTAGTG
2461	CTTTACGGCA	CCTCGACCCC	AAAAAACTTG	ATTAGGGTGA	TGGTTACAGT	AGTGGGCCAT
2521	CGCCCTGATA	GACGGTTTTT	CGCCCTTTGA	CGTTGGAGTC	CACGTTCTTT	AATAGTGGAC
2581	TCTTGTTCCA	AACTGGAACA	AACTCAACC	CTATCTCGGT	CTATTCTTTT	GATTTATAAG-

FIGURE 25C

2641 GGATTTTGCC GATTTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG  
2701 CGAATTTTAA CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC  
2761 GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAAA TATGTATCCG CTCATGAGAC  
2821 AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATTCAACATT  
2881 TCCGTGTGCG CCTTATTCCC TTTTTCGGG CATTTTGCCT TCCTGTTTTT GCTCACCCAG  
2941 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG  
3001 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTCG CCCGAAGAA CGTTTTCCAA  
3061 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGGC  
3121 AAGAGCAACT CGGTCGCCGC ATACACTATT CTCAGAATGA CTTGGTTGAG TACTCACCAG  
3181 TCACAGAAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT GCTGCCATAA  
3241 CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAAC GATCGGAGGA CCGAAGGAGC  
3301 TAACCGCTTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CTTGATCGT TGGGAACCGG  
3361 AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA GCAATGGCAA  
3421 CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA  
3481 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG  
3541 GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG  
3601 CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG  
3661 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT  
3721 GGTAACGTG AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCATTTTT  
3781 AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA ATCCCTTAAC  
3841 GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA TCTTCTTGAG  
3901 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG  
3961 TGGTTTGT TTGCCGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAGT GGCTTCAGCA  
4021 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  
4081 ACTCTGTAGC ACCGCTTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG GCTGCTGCCA  
4141 GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG GATAAGGCGC  
4201 AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA ACGACCTACA  
4261 CCGAAGTGA ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA  
4321 AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC  
4381 CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC TGACTTGAGC  
4441 GTCGATTTTT GTGATGCTCG TCAGGGGGGC GGAGCCTATG GAAAAACGCC AGCAACGCGG  
4501 CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTTGCTCA CATGTTCTTT CCTGCGTTAT  
4561 CCCCTGATTC TGTGGATAAC CGTATTACCG CTTTTGAGTG AGCTGATACC GCTCGCCGCA  
4621 GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC CCAATACGCA  
4681 AACC GCCTCT CCCC GCGCGT TGGCCGATTC ATTAATGCAG AGCTTGCAAT TCGCGCGCGA  
4741 AGGCGAAGCG GCATTTACGT TGACACCATC GAATGGCGCA AAACCTTTTCG CGGTATGGCA  
4801 TGATAGCGCC CGGAAGAGAG TCAATTCAGG GTGGTGAATG TGAAACCAGT AACGTTATAC  
4861 GATGTCGCAG AGTATGCCGG TGTCTCTTAT CAGACCGTTT CCCGCGTGGT GAACCAGGCC  
4921 AGCCACGTTT CTGCGAAAAC GCGGGAAAAA GTGGAAGCGG CGATGGCGGA GCTGAATTAC  
4981 ATTCCCAACC GCGTGGCACA ACAACTGGCG GGCAAACAGT CGTTGCTGAT TGGCGTTGCC  
5041 ACCTCCAGTC TGGCCCTGCA CGCGCCGTCG CAAATTGTCG CGGCGATTAA ATCTCGCGCC  
5101 GATCAACTGG GTGCCAGCGT GGTGGTGTG ATGGTAGAAC GAAGCGGCGT CGAAGCCTGT  
5161 AAAGCGGCGG TGCACAATCT TCTCGCGCAA CGGGTCAGTG GGCTGATCAT TAACTATCCG  
5221 CTGGATGACC AGGATGCCAT TGCTGTGGAA GCTGCCTGCA CTAATGTTCC GGC GTTATTT  
5281 CTTGATGTCT CTGACCAGAC ACCCATCAAC AGTATTATTT TCTCCCATGA AGACGGTACG  
5341 CGACTGGGCG TGGAGCATCT GGTGCGATTG GGTCAACAGC AAATCGCGCT GTTAGCGGGC  
5401 CCATTAAGTT CTGTCTCGGC GCGTCTGCGT CTGGCTGGCT GGCATAAATA TCTCACTCGC  
5461 AATCAAATTC AGCCGATAGC GGAACGGGAA GGCGACTGGA GTGCCATGTC CGGTTTTCAA  
5521 CAAACCATGC AAATGCTGAA TGAGGGCATC GTTCCCACTG CGATGCTGGT TGCCAACGAT  
5581 CAGATGGCGC TGGGCGCAAT GCGCGCCATT ACCGAGTCCG GGCTGCGCGT TGGTGC GGAT  
5641 ATCTCGGTAG TGGGATACGA CGATACCGAA GACAGCTCAT GTTATATCCC GCCGTCAACC  
5701 ACCATCAAAC AGGATTTTCG CCTGCTGGGG CAAACCAGCG TGGACCGCTT GCTGCAACTC  
5761 TCTCAGGGCC AGGCGGTGAA GGGCAATCAG CTGTTGCCCG TCTCACTGGT GAAAAGAAAA  
5821 ACCACCCTGG CGCCCAATAC GCAAACCGCC TCTCCCGCG CGTTGGCCGA TTCATTAATG  
5881 CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT  
5941 GAGTTAGCTC ACTCATT

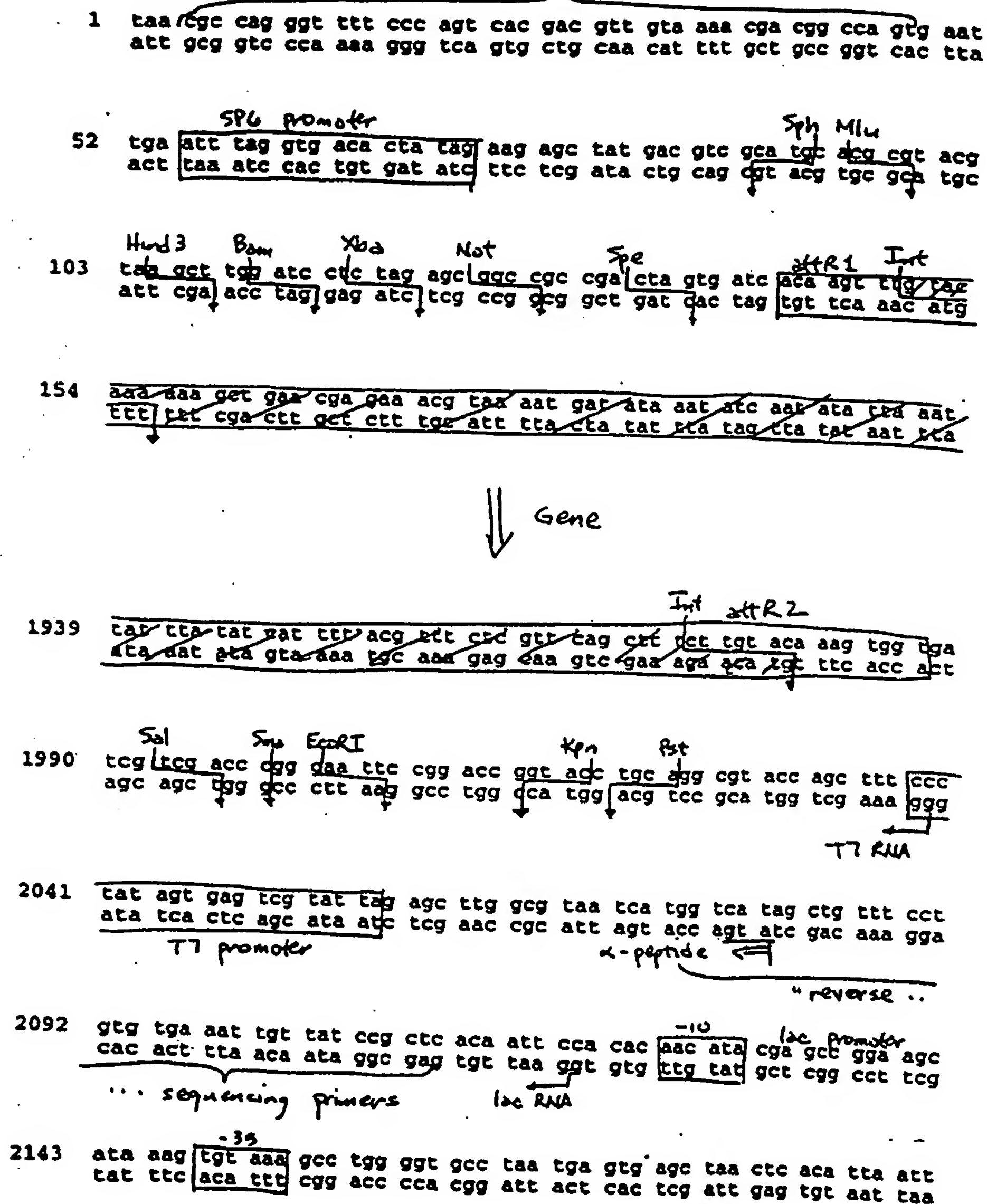
FIGURE 25D

Figure 24A

pDEST6

pSPORT "..."  
(opposite strand)

"forward" sequencing primers

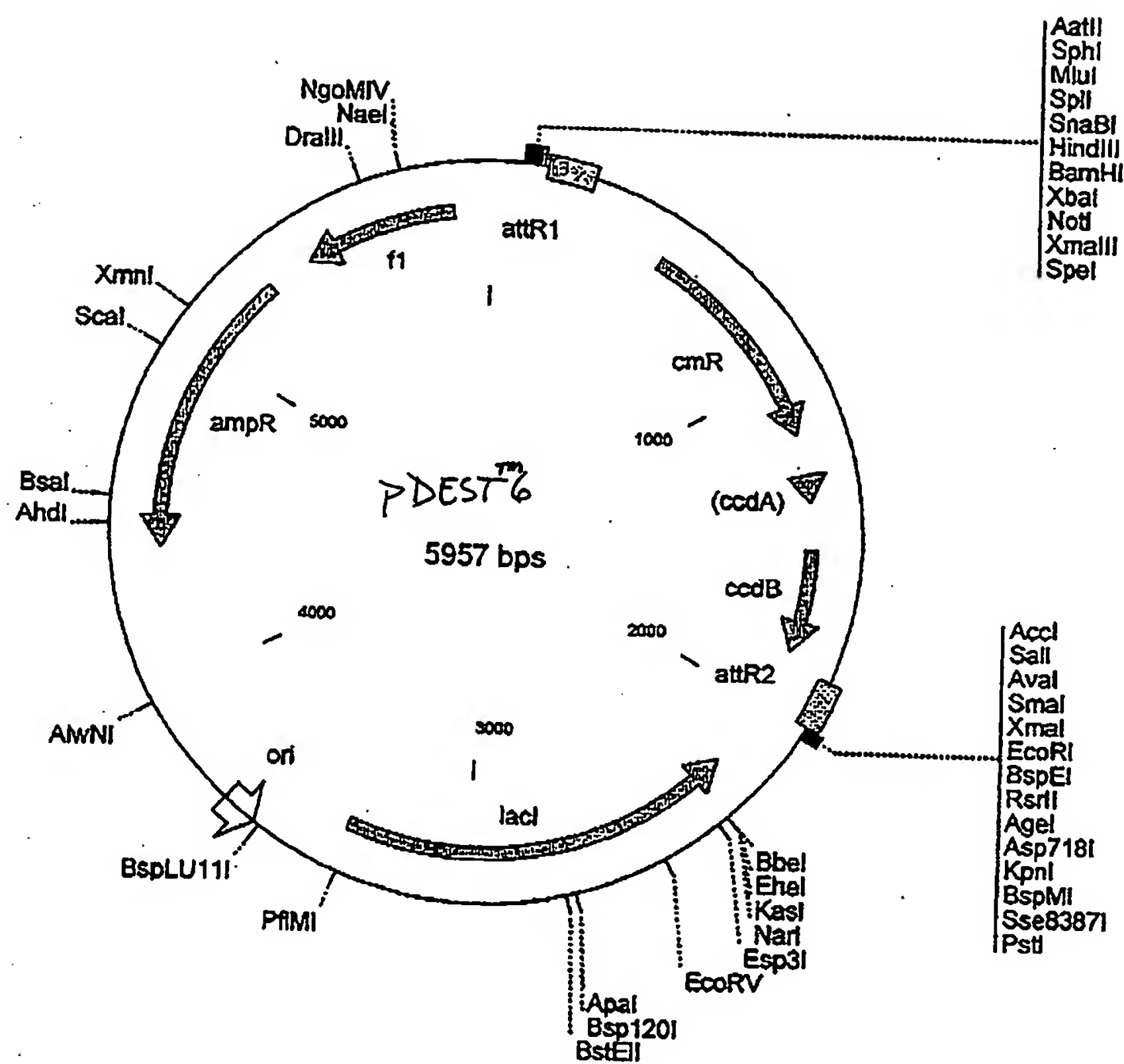


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Figure 26B

PDEST6

(cont'd)



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## pDEST6 5957 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
266..142		attR1
516..1175		CmR
1295..1379		inactivated ccdA
1517..1822		ccdB
1863..1987		attR2
2203..3369		lacI
4403..5260		ampR
5392..5847		f1 (f1 intergenic region)

1	TAACGCCAGG	GTTTTCCCAG	TCACGACGTT	GTAAAACGAC	GGCCAGTGAA	TTGAATTTAG
61	GTGACACTAT	AGAAGAGCTA	TGACGTCGCA	TGCACGCGTA	CGTAAGCTTG	GATCCTCTAG
121	AGCGGCCGCC	GACTAGTGAT	CACAAGTTTG	TACAAAAAAG	CTGAACGAGA	AACGTAAAT
181	GATATAAATA	TCAATATATT	AAATTAGATT	TTGCATAAAA	AACAGACTAC	ATAATACTGT
241	AAAACACAAC	ATATCCAGTC	ACTATGGCGG	CCGCTAAGTT	GGCAGCATCA	CCCGACGCAC
301	TTTGCGCCGA	ATAAATACCT	GTGACGGAAG	ATCACTTCGC	AGAATAAATA	AATCCTGGTG
361	TCCCTGTTGA	TACCGGGAAG	CCCTGGGCCA	ACTTTTGGCG	AAAATGAGAC	GTTGATCGGC
421	ACGTAAGAGG	TTCCAACTTT	CACCATAATG	AAATAAGATC	ACTACCGGGC	GTATTTTTTG
481	AGTTATCGAG	ATTTTCAGGA	GCTAAGGAAG	CTAAAATGGA	GAAAAAATC	ACTGGATATA
541	CCACCGTTGA	TATATCCCAA	TGGCATCGTA	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG
601	CTCAATGTAC	CTATAACCAG	ACCGTTCAGC	TGGATATTAC	GGCCTTTTTA	AAGACCGTAA
661	AGAAAAATAA	GCACAAGTTT	TATCCGGCCT	TTATTCACAT	TCTTGCCCGC	CTGATGAATG
721	CTCATCCGGA	ATTCCGTATG	GCAATGAAAG	ACGGTGAGCT	GGTGATATGG	GATAGTGTTT
781	ACCCTTGTTA	CACCGTTTTT	CATGAGCAAA	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT
841	ACCACGACGA	TTTCCGGCAG	TTTCTACACA	TATATTGCGA	AGATGTGGCG	TGTTACGGTG
901	AAAACCTGGC	CTATTTCCCT	AAAGGGTTTA	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC
961	CCTGGGTGAG	TTTCACCACT	TTTGATTAA	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC
1021	CCGTTTTTAC	CATGGGCAAA	TATTATACGC	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA
1081	TTCAGGTTCA	TCATGCCGTC	TGTGATGGCT	TCCATGTCCG	CAGAATGCTT	AATGAATTAC
1141	AACAGTACTG	CGATGAGTGG	CAGGGCGGGG	CGTAAACGCG	TGGATCCGGC	TTACTAAAAG
1201	CCAGATAACA	GTATGCGTAT	TTGCGCGCTG	ATTTTTGCGG	TATAAGAATA	TATACTGATA
1261	TGTATACCCG	AAGTATGTCA	AAAAGAGGTG	TGCTATGAAG	CAGCGTATTA	CAGTGACAGT
1321	TGACAGCGAC	AGCTATCAGT	TGCTCAAGGC	ATATATGATG	TCAATATCTC	CGGTCTGGTA
1381	AGCACAACCA	TGCAGAAATG	AGCCCGTCGT	CTGCGTGCCG	AACGCTGGAA	AGCGGAAAAT
1441	CAGGAAGGGA	TGGCTGAGGT	CGCCCGGTTT	ATTGAAATGA	ACGGCTCTTT	TGCTGACGAG
1501	AACAGGGACT	GGTGAATGTC	AGTTTAAAGT	TTACACCTAT	AAAAGAGAGA	GCCGTTATCG
1561	TCTGTTTGTG	GATGTACAGA	GTGATATTAT	TGACACGCCC	GGGCGACGGA	TGGTGATCCC
1621	CCTGGCCAGT	GCACGTCTGC	TGTCAGATAA	AGTCTCCCGT	GAACTTTACC	CGGTGGTGCA
1681	TATCGGGGAT	GAAAGCTGGC	GCATGATGAC	CACCGATATG	GCCAGTGTGC	CGGTCTCCGT
1741	TATCGGGGAA	GAAAGTGGCTG	ATCTCAGCCA	CCGCGAAAAT	GACATCAAAA	ACGCCATTAA
1801	CCTGATGTTT	TGGGGAATAT	AAATGTCAGG	CTCCCTTATA	CACAGCCAGT	CTGCAGGTCG
1861	ACCATAGTGA	CTGGATATGT	TGTGTTTTAC	AGTATTATGT	AGTCTGTTTT	TTATGCAAAA
1921	TCTAATTTAA	TATATTGATA	TTTATATCAT	TTTACGTTTC	TCGTTTCAGCT	TTCTTGATCA
1981	AAGTGGTGAT	CGTCGACCCG	GGAATTCCGG	ACCGGTACCT	GCAGGCGTAC	CAGCTTTCCC
2041	TATAGTGAGT	CGTATTAGAG	CTTGGCGTAA	TCATGGTCAT	AGCTGTTTCC	TGTGTGAAAT
2101	TGTTATCCGC	TCACAATTCC	ACACAACATA	CGAGCCGGAA	GCATAAAGTG	TAAAGCCTGG
2161	GGTGCCTAAT	GAGTGAGCTA	ACTCACATTA	ATTGCGTTGC	GCTCACTGCC	CGCTTTCCAG
2221	TCGGGAAACC	TGTCGTGCCA	GCTGCATTAA	TGAATCGGCC	AACGCGCGGG	GAGAGGCGGT
2281	TTGCGTATTG	GGCGCCAGGG	TGGTTTTTCT	TTTCACCAGT	GAGACGGGCA	ACAGCTGATT
2341	GCCCTTCACC	GCCTGGCCCT	GAGAGAGTTG	CAGCAAGCGG	TCCACGCTGG	TTTGCCCCAG
2401	CAGGCGAAAA	TCCTGTTTGA	TGGTGGTTGA	CGGCGGGATA	TAACATGAGC	TGTCTTCCGT
2461	ATCGTCGTAT	CCCACTACCG	AGATATCCGC	ACCAACGCGC	AGCCCGGACT	CGGTAATGGC
2521	GCGCATTGCG	CCCAGCGCCA	TCTGATCGTT	GGCAACCAGC	ATCGCAGTGG	GAACGATGCC
2581	CTCATTCAGC	ATTGTCATGG	TTTGTTGAAA	ACCGGACATG	GCACTCCAGT	CGCCTTCCCC
2641	TTCCGCTATC	GGCTGAATTT	GATTGCGAGT	GAGATATTTA	TGCCAGCCAG	CCAGACGCAG-

FIGURE 26C



2701 ACGCGCCGAG ACAGAACTTA ATGGGCCCCG TAACAGCGCG ATTTGCTGGT GACCCAATGC  
2761 GACCAGATGC TCCACGCCCA GTCGCGTACC GTCTTCATGG GAGAAAATAA TACTGTTGAT  
2821 GGGTGTCTGG TCAGAGACAT CAAGAAATAA CGCCGGAACA TTAGTGCAGG CAGCTTCCAC  
2881 AGCAATGGCA TCCTGGTCAT CCAGCGGATA GTTAATGATC AGCCCACTGA CCCGTTGCGC  
2941 GAGAAGATTG TGCACCGCCG CTTTACAGGC TTCGACGCCG CTTGCTTCTA CCATCGACAC  
3001 CACCACGCTG GCACCCAGTT GATCGGCGCG AGATTTAATC GCCGCGACAA TTTGCGACGG  
3061 CGCGTGCAGG GCCAGACTGG AGGTGGCAAC GCCAATCAGC AACGACTGTT TGCCCGCCAG  
3121 TTGTTGTGCC ACGCGGTTGG GAATGTAATT CAGCTCCGCC ATCGCCGCTT CCACTTTTTC  
3181 CCGCGTTTTTC GCAGAAACGT GGCTGGCCTG GTTCACCACG CGGGAAACGG TCTGATAAGA  
3241 GACACCGGCA TACTCTGCGA CATCGTATAA CGTTACTGGT TTCACATTCA CCACCCTGAA  
3301 TTGACTCTCT TCCGGGCGCT ATCATGCCAT ACCGCGAAAG GTTTTGCGCC ATTCGATGGT  
3361 GTCAACGTAA ATGCCGCTTC GCCTTCGCGC GCGAATTGCA AGCTCTGCAT TAATGAATCG  
3421 GCCAACGCGC GGGGAGAGGC GGTTTTCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG  
3481 ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA  
3541 TACGTTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC  
3601 AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC  
3661 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT  
3721 AAAGATACCA GCGGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC  
3781 CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTGCGGAAG CGTGGCGCTT TCTCAATGCT  
3841 CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG  
3901 AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC  
3961 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA  
4021 GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACACGGC TACACTAGAA  
4081 GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTGCGAAAA AGAGTTGGTA  
4141 GCTCTTGATC CGGCAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC  
4201 AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG  
4261 ACGCTCAGTG GAACGAAAAC TCACGTAAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA  
4321 TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTAA ATCAATCTAA AGTATATATG  
4381 AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT  
4441 GTCTATTTTCG TTCATCCATA GTTGCCCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG  
4501 AGGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC  
4561 CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCTGCAA  
4621 CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC  
4681 CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG TCACGCTCGT  
4741 CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC  
4801 CCATGTTGTG CAAAAAAGCG GTTAGCTCCT TCGGTCTCTC GATCGTTGTC AGAAGTAAGT  
4861 TGGCCGCACT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC  
4921 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT  
4981 GTATGCGGCG ACCGAGTTGC TCTTGCCCCG CGTCAATACG GGATAATACC GCGCCACATA  
5041 GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA  
5101 TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCTCTCG TGCACCCAAC TGATCTTCAG  
5161 CATCTTTTAC TTTCAACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA  
5221 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT  
5281 ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA  
5341 AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT GAAATTGTAA  
5401 ACGTTAATAT TTTGTTAAAA TTCGCGTTAA ATTTTGTGTA AATCAGCTCA TTTTTTAACC  
5461 AATAGGCCGA AATCGGCAAA ATCCCTTATA AATCAAAAAGA ATAGACCGAG ATAGGGTTGA  
5521 GTGTTGTTCC AGTTTGGAAC AAGAGTCCAC TATTAAAGAA CGTGGACTCC AACGTCAAAG  
5581 GCGAAAAAAC CGTCTATCAG GGCGATGGCC CACTACGTGA ACCATCACCC TAATCAAGTT  
5641 TTTTGGGGTC GAGGTGCCGT AAAGCACTAA ATCGGAACCC TAAAGGGAGC CCCCATTATA  
5701 GAGCTTGACG GGGAAAGCCG GCGAACGTGG CGAGAAAGGA AGGGAAGAAA GCGAAAGGAG  
5761 CGGGCGCTAG GGCGCTGGCA AGTGTAGCGG TCACGCTGCG CGTAACCACC ACACCCGCCG  
5821 CGCTTAATGC GCCGCTACAG GGCGCGTCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA  
5881 AGGGCGATCG GTGCGGGCCT CTTGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC  
5941 AAGGCGATTA AGTTGGG

FIGURE 26D

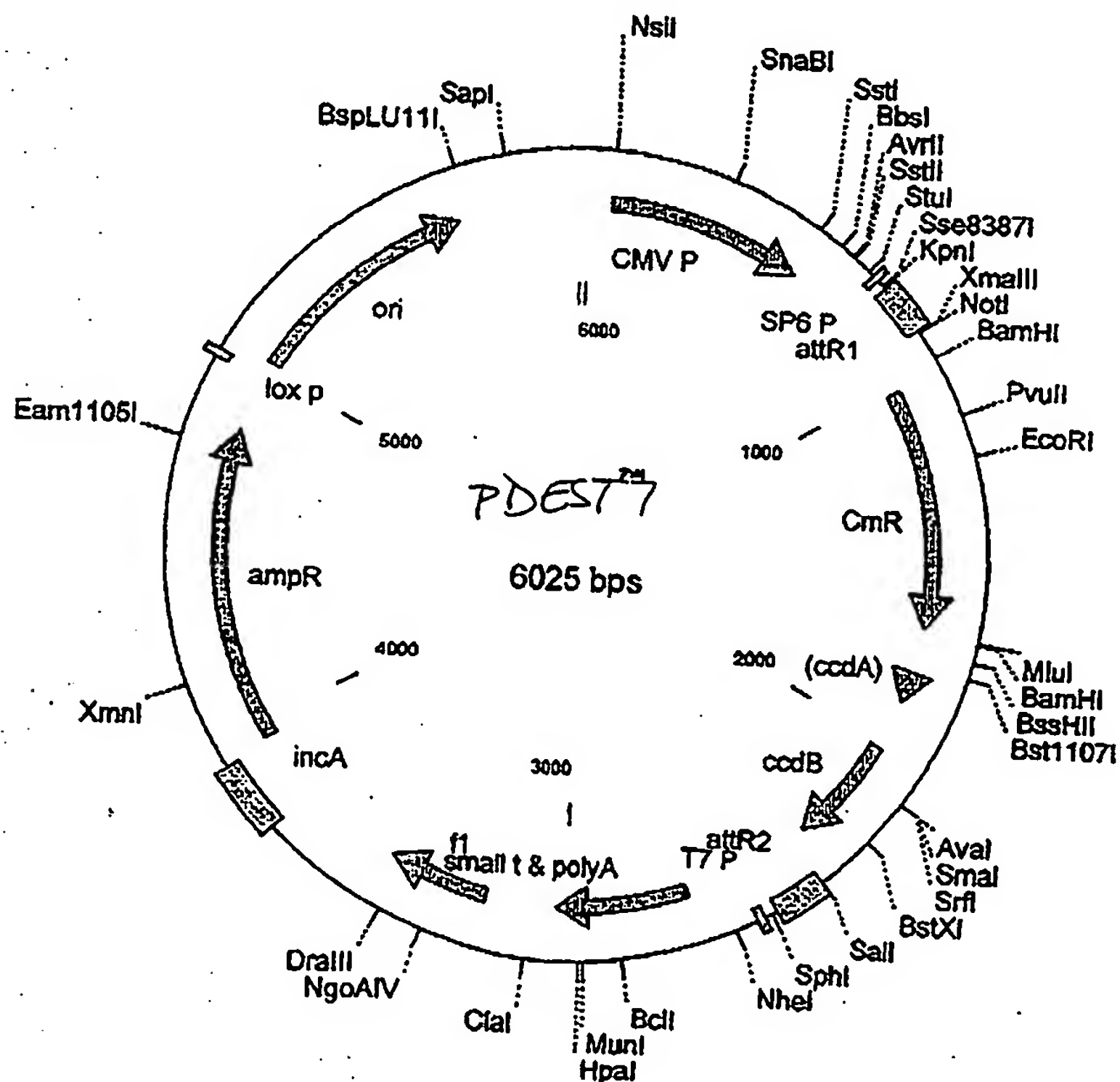


Figure 2A: PDEST7

## CMV promoter for eukaryotic expression

970 cca ttg acg caa atg ggc ggt agg cgt gta cgg tgg gag gtc tat ata agc  
 ggt aac tgc gtt tac ccg cca tcc gca cat gcc acc ctc cag ata tat tcc  
 1021 aga gct cgt tta gtg aac cgt cag atc gcc tgg aga cgc cat cca cgc tgt  
 tct cga gca aat cac ttg gca gtc tag cgg acc tct gcg gta ggt gcg aca  
 1072 ttt gac ctc cat aga aga cac cgg gac cga tcc agc ctc cgg act cta gcc  
 aaa ctg gag gta tct tct gtg gcc ctg gct agg tcc gag gcc tga gat cgg  
 1123 tag gcc gcg gag cgg ata aca att tca cac agg aaa cag cta tga cca cta  
 atc cgg cgc ctc gcc tat tgt taa agt gtg tcc ttt gtc gat act ggt gat  
 1174 ggc ttt tgc aaa aag cta ttt agg tga cac tat aga agg tac gcc tgc agg  
 ccg aaa acg ttt ttc gat aaa tcc act gtg ata tct tcc atg cgg acg tcc  
 1225 tac cgg tcc gga att ccc atc aca agt tgg tag aac aac ggt gaa cga gaa  
 atg gcc agg cct taa ggg tag tgt tca aac atg ttt ttt tga ctc gct ctc

mRNA start  
 CMV enhancer/promoter  
 Pst  
 Kpn  
 EcoRI  
 attR1



## pDEST7 6025 bp (rotated to position 2800)

Location (Base Nos.)	Gene Encoded
67..589	CMV promoter
906..782	attR1
1015..1674	CmR
1794..1878	inactivated ccdA
2016..2321	ccdB
2362..2486	attR2
2671..3033	small t & polyA
3227..3502	f1
3962..4822	ampR
5022..5661	ori

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1 ATTATCATGA CATTAACTTA TAAAAATAGG CGTAGTACGA GGCCCTTTCA CTCATTAGAT
61 GCATGTCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA ACGACCCCCG
121 CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG
181 ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA
241 TATGCCAAGT ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC
301 CCAGTACATG ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC
361 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
421 ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA
481 TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA TTGACGCAAA TGGGCGGTAG
541 GCGTGTACGG TGGGAGGTCT ATATAAGCAG AGCTCGTTTA GTGAACCGTC AGATCGCCTG
601 GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCG
661 GACTCTAGCC TAGGCCGCGG AGCGGATAAC AATTTACAC AGGAAACAGC TATGACCATT
721 AGGCCTTTGC AAAAAGCTAT TTAGGTGACA CTATAGAAGG TACGCCTGCA GGTACCGGAT
781 CACAAGTTTG TACAAAAAAG CTGAACGAGA AACGTAAAAAT GATATAAATA TCAATATATT
841 AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT AAAACACAAC ATATCCAGTC
901 ACTATGGCGG CCGCATTAGG CACCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATAAT
961 GTGTGGATTT TGAGTTAGGA TCCGTCGAGA TTTTCAGGAG CTAAGGAAGC TAAAATGGAG
1021 AAAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA AGAACATTTT
1081 GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT GGATATTACG
1141 GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTTT ATCCGGCCTT TATTCACATT
1201 CTTGCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG CAATGAAAGA CCGTGAGCTG
1261 GTGATATGGG ATAGTGTTC A CCTTGTTC ACCGTTTTC ATGAGCAAAC TGAAACGTTT
1321 TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT ATATTCGCAA
1381 GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT TGAGAATATG
1441 TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAGT TTGATTTAAA CGTGGCCAAT
1501 ATGGACAAC TCTTCGCCCC CGTTTTCAAC ATGGGCAAAT ATTATACGCA AGGCGACAAG
1561 GTGCTGATGC CGCTGGCGAT TCAGGTTTCAT CATGCCGTCT GTGATGGCTT CCATGTCGGC
1621 AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC GTAAACGCGT
1681 GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA TTTTTCGGGT
1741 ATAAGAATAT ATACTGATAT GTATACCCGA AGTATGTCAA AAAGAGGTGT GCTATGAAGC
1801 AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA TATATGATGT
1861 CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA GCCCGTCGTC TGCGTGCCGA
1921 ACGCTGGAAA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCCCGGTTTA TTGAAATGAA
1981 CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT TACACCTATA
2041 AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT GACACGCCCC
2101 GGCGACGGAT GGTGATCCCC CTGGCCAGTG CACGTCTGCT GTCAGATAAA GTCTCCCGTG
2161 AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC ACCGATATGG
2221 CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC CGCGAAAATG
2281 ACATCAAAAA CGCCATTAACT CTGATGTTCT GGGGAATATA AATGTCAGGC TCCCTTATAC
2341 ACAGCCAGTC TGCAGGTGCA CCATAGTGAC TGGATATGTT GTGTTTTACA GTATTATGTA
2401 GTCTGTTTTT TATGCAAAAT CTAATTTAAT ATATTGATAT TTATATCATT TTACGTTTCT
2461 CGTTCAGCTT TCTGTACAA AGTGGTGATC GCGTGATGC GACGTCATAG CTCTCTCCCT
2521 ATAGTGAGTC GTATTATAAG CTAGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA-

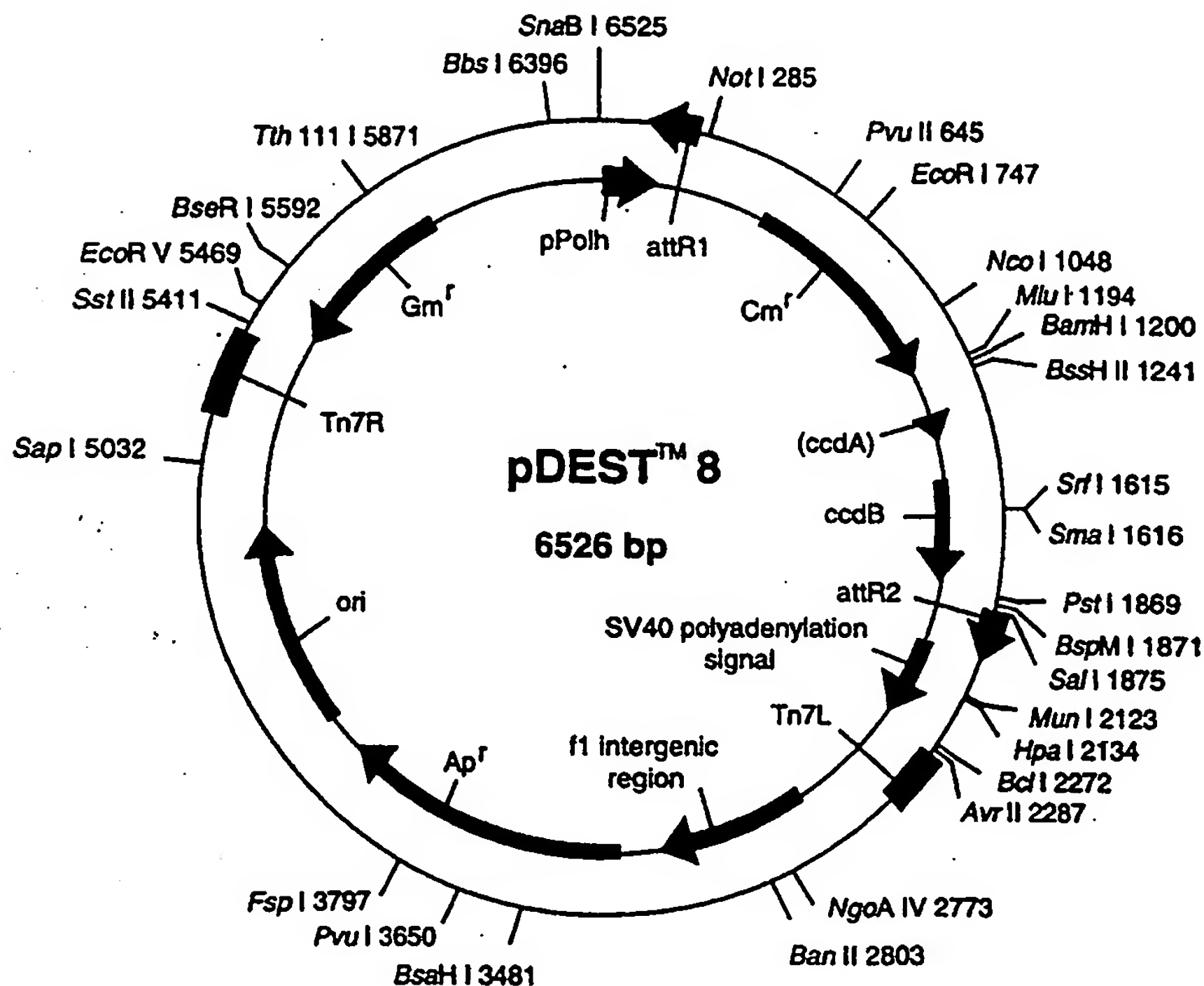
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2581 AACTGCTAGC TTGGGATCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC ATAATTGGAC  
2641 AAACCTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA AATTTTAAAG TGTATAATGT  
2701 GTTAAACTAG CTGCATATGC TTGCTGCTTG AGAGTTTTCG TTAGTGAGTA TGATTTATGA  
2761 AAATATTATA CACAGGAGCT AGTGATTCTA ATTGTTTGTG TATTTTAGAT TCACAGTCCC  
2821 AAGGCTCATT TCAGGCCCTT CAGTCCTCAC AGTCTGTTCA TGATCATAAT CAGCCATACC  
2881 ACATTTGTAG AGGTTTTACT TGCTTTAAAA AACCTCCCAC ACCTCCCCCT GAACCTGAAA  
2941 CATAAAATGA ATGCAATTGT TGTGTGTTAA TTGTTTATTG CAGCTTATAA TGGTTACAAA  
3001 TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCAGTGCA TTCTAGTTGT  
3061 GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCCTGC ATTAATGAAT  
3121 CGGCCAACGC GCGGGGAGAG GCGGTTTTCG TATTGGCTGG CGTAATAGCG AAGAGGCCCCG  
3181 CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGGACG CGCCCTGTAG  
3241 CGGCGCATTG AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG  
3301 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCTTCCCTT CTGCCCACGT TCGCCGGCTT  
3361 TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC CGATTTAGTG CTTTACGGCA  
3421 CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA  
3481 GACGGTTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCTA  
3541 AACTGGAACA AACTCAACC CTATCTCGGT CTATCTTTT GATTTATAAG GGATTTTGCC  
3601 GATTTGCGCC TATTGGTTAA AAAATGAGCT GATTTAACA AAATTTAACG CGAATTTTAA  
3661 CAAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC  
3721 TATTTGTTTA TTTTCTAAA TACATTCAAA TATGTATCCG CTCATGCCAG GTCTTGGAAT  
3781 GGTGAGAACG GCTTGCTCGG CAGCTTCGAT GTGTGCTGGA GGGAGAATAA AGGTCTAAGA  
3841 TGTGCGATAG AGGGAAGTCG CATTGAATTA TGTGCTGTGT AGGGATCGCT GGTATCAAAT  
3901 ATGTGTGCCC ACCCCTGGCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA  
3961 AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT  
4021 TTGCCCTTCT GTTTTGTCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA  
4081 GTTGGGTGCA CGAGTGGGT ACATCGAAGT GGATCTCAAC AGCGGTAAAG TCCTTGAGAG  
4141 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC  
4201 GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA  
4261 GAATGACTTG GTTGAGTACT CACCACTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT  
4321 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT  
4381 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTCG CACAACATGG GGGATCATGT  
4441 AACTCGCCTT GATCGTTGGG AACCAGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA  
4501 CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAAGTG GCGAACTACT  
4561 TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC  
4621 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA  
4681 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT  
4741 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA  
4801 GATAGGTGCC TCACTGATTA AGCATTTGTA ACTGTCAGAC CAAGTTTACT CATATATACT  
4861 TTAGATTGAT TTAAACTTTC ATTTTAAATT TAAAGGATC TAGGTGAAGA TCCTTTTGA  
4921 TAATCTCATG CCATAACTTC GTATAATGTA TGCTATACGA AGTTATGGCA TGACCAAAAT  
4981 CCTTAAACGT GAGTTTTCTG TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
5041 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
5101 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAAGTGG  
5161 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCTAGTG TAGCCGTAGT TAGGCCACCA  
5221 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCTAGTG TAGCCGTAGT TAGGCCACCA  
5281 TGCTGCCAGT GCGGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
5341 TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TCGTGACACA CAGCCCAGCT TGGAGCGAAC  
5401 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTTA GAAAGCGCCA CGCTTCCCGA  
5461 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG  
5521 GGAGCTTCCA GGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
5581 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
5641 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTCTTTTCC  
5701 TCGGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
5761 TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC  
5821 AATACGCAA CCGCCTCTCC CCGCGCTTTC GCCGATTCAT TAATGCAGAG CTTGCAATTC  
5881 GCGCGTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT  
5941 ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTT CCCGAAAAGT  
6001 GCCACCTGAC GTCTAAGAAA CCATT

Figure 28A: pDEST8 Polyhedron Promoter, Baculovirus Transfer Plasmid

AccI  
 1 cgt|ata ctc cgg aat att aat aga tca tgg aga taa tta aaa tga taa cca  
 gca tat gag gcc tta taa tta tct agt acc tct att aat ttt act att ggt  
 52 tct cgc aaa taa ata agt att tta ctg ttt tgc taa cag ttt tgt aat aaa  
 aga gcg ttt att tat tca taa aat gac aaa agc att gtc aaa aca tta ttt  
 103 aaa acc tat aaa tat tcc gga tta ttc ata ccg tcc cac cat cgg gcg cgg  
 ttt tgg ata ttt ata agg cct aat aag tat ggc agg gtg gta gcc cgc gcc  
 154 atc atc aca agt tgg tag aca aaa gct gaa cga gaa agg taa dat gat ata  
 tag tag tgt tca aac atg ttt ttc cga ctt gct ctt tgc att tta cta tat

mRNA (polyhedrin)  
 T<sub>AT</sub> attR1





60/240

## pDEST8 6526 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
23..152		Ppolh
284..160		attR1
534..1193		CmR
1313..1397		inactivated ccdA
1535..1840		ccdB
1881..2005		attR2
2766..3146		f1
3240..4090		ampR
4289..4869		ori
5564..6496		genR

1	CGTATACTCC	GGAATATTAA	TAGATCATGG	AGATAATTAA	AATGATAACC	ATCTCGCAAA
61	TAAATAAGTA	TTTTACTGTT	TTCGTAACAG	TTTTGTAATA	AAAAAACCTA	TAAATATTCC
121	GGATTATTCA	TACCGTCCCA	CCATCGGGCG	CGGATCATCA	CAAGTTTGTA	CAAAAAAGCT
181	GAACGAGAAA	CGTAAAATGA	TATAAATATC	AATATATTAA	ATTAGATTTT	GCATAAAAAA
241	CAGACTACAT	AATACTGTAA	AACACAACAT	ATCCAGTCAC	TATGGCGGCC	GCTAAGTTGG
301	CAGCATCACC	CGACGCACTT	TGCGCCGAAT	AAATACCTGT	GACGGAAGAT	CACTTCGCAG
361	AATAAATAAA	TCCTGGTGTG	CCTGTTGATA	CCGGGAAGCC	CTGGGCCAAC	TTTTGGCGAA
421	AATGAGACGT	TGATCGGCAC	GTAAGAGGTT	CCAACTTTCA	CCATAATGAA	ATAAGATCAC
481	TACCGGGCGT	ATTTTTTGAG	TTATCGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA
541	AAAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG
601	AGGCATTTCA	GTCAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG
661	CCTTTTTTAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTCACATTC
721	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG
781	TGATATGGGA	TAGTGTTTCA	CCTTGTTACA	CCGTTTTTCA	TGAGCAAACT	GAAACGTTTT
841	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTCGCAAG
901	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT
961	TTTTTCGTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC	GTGGCCAATA
1021	TGGACAACCT	CTTCGCCCCC	GTTTTTACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG
1081	TGCTGATGCC	GCTGGCGATT	CAGGTTTCATC	ATGCCGCTCTG	TGATGGCTTC	CATGTCGGCA
1141	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG
1201	GATCCGGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA
1261	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA
1321	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC
1381	AATATCTCCG	GTCTGGTAAG	CACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA
1441	CGCTGGAAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTCG	CCCGGTTTAT	TGAAATGAAC
1501	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA
1561	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATTG	ACACGCCCCG
1621	GCGACGGATG	GTGATCCCCC	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA
1681	ACTTTACCCG	GTGGTGCATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC
1741	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA
1801	CATCAAAAAC	GCCATTAAAC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA
1861	CAGCCAGTCT	GCAGGTCGAC	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG
1921	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA	TATTGATATT	TATATCATTT	TACGTTTCTC
1981	GTTTCAGCTTT	CTTGTAACAA	GTGGTGATAG	CTTGTCGAGA	AGTACTAGAG	GATCATAATC
2041	AGCCATACCA	CATTTGTAGA	GGTTTTACTT	GCTTTAAAAA	ACCTCCCACA	CCTCCCCCTG
2101	AACCTGAAAC	ATAAAATGAA	TGCAATTGTT	GTTGTTAACT	TGTTTATTGC	AGCTTATAAT
2161	GGTTACAAAT	AAAGCAATAG	CATCACAAAT	TTCACAAATA	AAGCATTTTT	TTCATGTCAT
2221	TCTAGTTGTG	GTTTGTCCAA	ACTCATCAAT	GTATCTTATC	ATGTCTGGAT	CTGATCACTG
2281	CTTGAGCCTA	GGAGATCCGA	ACCAGATAAG	TGAAATCTAG	TTCCAAACTA	TTTTGTTCATT
2341	TTTAATTTTC	GTATTAGCTT	ACGACGCTAC	ACCCAGTTCC	CATCTATTTT	GTCATCTTTC
2401	CCTAAATAAT	CCTTAAAAAC	TCCATTTCCA	CCCCTCCCAG	TTCCCAACTA	TTTTGTCCGC
2461	CCACAGCGGG	GCATTTTTCT	TCCTGTTATG	TTTTTAATCA	AACATCCTGC	CAACTCCATG
2521	TGACAAACCG	TCATCTTCGG	CTACTTTTTT	TCTGTCACAG	AATGAAAATT	TTTCTGTCAT

FIGURE 28B

2581 CTCTTCGTTA TTAATGTTTG TAATTGACTG AATATCAACG CTTATTTGCA GCCTGAATGG  
2641 CGAATGGACG CGCCCTGTAG CGGCGCATT AAGCGCGCGG GTGTGGTGGT TACGCGCAGC  
2701 GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCTTCCTTT  
2761 CTCGCCACGT TCGCCGGCTT TCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC  
2821 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAACTTG ATTAGGGTGA TGGTTCACGT  
2881 AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC CACGTTCTTT  
2941 AATAGTGGAC TCTTGTTCCA AACTGGAACA AACTCAACC CTATCTCGGT CTATCTTTT  
3001 GATTTATAAG GGATTTTGCC GATTTCCGCC TATTGGTTAA AAAATGAGCT GATTTAACAA  
3061 AAATTTAACG CGAATTTTAA CAAATATTA ACGTTTACAA TTTCAGGTGG CACTTTTCGG  
3121 GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTCTAAA TACATTCAA TATGTATCCG  
3181 CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT  
3241 ATTCAACATT TCCGTGTCGC CTTATTCCC TTTTTCGCG CATTTTGCCT TCCTGTTTTT  
3301 GCTCACCAG AAACGCTGGT GAAAGTAAA GATGCTGAAG ATCAGTTGGG TGCACGAGT  
3361 GGTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTTCG CCCCAGAGAA  
3421 CGTTTTCCAA TGATGAGCAC TTTTAAAGT CTGCTATGT GCGCGGTATT ATCCCGTATT  
3481 GACGCCGGG AAGAGCAACT CGGTGCGCG ATACACTATT CTCAGAATGA CTTGGTTGAG  
3541 TACTCACCAG TCACAGAAA GCATCTTACG GATGGCATGA CAGTAAGAGA ATTATGCAGT  
3601 GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC TTCTGACAA GATCGGAGGA  
3661 CCGAAGGAGC TAACCGCTT TTTGCACAAC ATGGGGGATC ATGTAACCTG CTTTGATCGT  
3721 TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC GTGACACCAC GATGCCTGTA  
3781 GCAATGGCAA CAACGTTGCG CAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG  
3841 CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC  
3901 CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT  
3961 ATCATTGCAG CACTGGGGCC AGATGGTAAG CCTCCCGTA TCGTAGTTAT CTACACGACG  
4021 GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG  
4081 ATTAAGCATT GGTAAGTGC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA  
4141 CTTCATTTTT AATTTAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA  
4201 ATCCCTTAAC GTGAGTTTTT GTTCCACTGA GCGTCAGACC CCGTAGAAA GATCAAAGGA  
4261 TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG  
4321 CTACCAGCGG TGGTTTGTG GCCGGATCAA GAGCTACCAA CTCTTTTTCC GAAGGTAAGT  
4381 GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC  
4441 CACTTCAAGA ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG  
4501 GCTGCTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTTGG ACTCAAGACG ATAGTTACCG  
4561 GATAAGGCGC AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCCAG CTTGGAGCGA  
4621 ACGACCTACA CCGAAGTGA ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC  
4681 GAAGGGAGAA AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCAGG  
4741 AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGCGGTT TCGCCACCTC  
4801 TGACTTGAGC GTCGATTTTT GTGATGCTCG TCAGGGGGG GGAGCCTATG GAAAAACGCC  
4861 AGCAACGCGG CCTTTTTACG GTTCTGGGCC TTTTGTGCG CTTTTGCTCA CATGTTCTTT  
4921 CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC  
4981 GCTCGCCGCA GCCGAACGAC CGAGCGCAGC GAGTCAGTGA GCGAGGAAGC GGAAGAGCGC  
5041 CTGATGCGGT ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAG ACCAGCCGCG  
5101 TAACCTGGCA AAATCGGTTA CGGTTGAGTA ATAAATGGAT GCCCTGCGTA AGCGGGTGTG  
5161 GGCGGACAAT AAAGTCTTAA ACTGAACAAA ATAGATCTAA ACTATGACAA TAAAGTCTTA  
5221 AACTAGACAG AATAGTTGTA AACTGAAATC AGTCCAGTTA TGCTGTGAAA AAGCATACTG  
5281 GACTTTTGTT ATGGCTAAAG CAACTCTTC ATTTTCTGAA GTGCAAATTG CCCGTCTGAT  
5341 TAAAGAGGGG CGTGGCCAAG GGCATGGTAA AGACTATATT CGCGGCGTTG TGACAATTTA  
5401 CCGAACAAC CCGCGGCCG GAAGCCGATC TCGGCTTGAA CGAATTGTTA GGTGGCGGTA  
5461 CTTGGGTCGA TATCAAAGTG CATCACTTCT TCCCGTATGC CCAACTTGT ATAGAGAGCC  
5521 ACTGCGGGAT CGTACCCTGA ATCTGCTTGC ACGTAGATCA CATAAGCACC AAGCGCGTTG  
5581 GCCTCATGCT TGAGGAGATT GATGAGCGCG GTGGCAATGC CCTGCCTCCG GTGCTCGCCG  
5641 GAGACTGCGA GATCATAGAT ATAGATCTCA CTACGCGGCT GCTCAAACCT GGGCAGAACG  
5701 TAAGCCGCGA GAGCGCCAAC AACCGCTTCT TGGTCGAAGG CAGCAAGCGC GATGAATGTC  
5761 TTAACCGGA GCAAGTTCCC GAGGTAATCG GAGTCCGGCT GATGTTGGGA GTAGGTGGCT  
5821 ACGTCTCCGA ACTCAGACC GAAAGATCA AGAGCAGCCC GCATGGATTT GACTTGGTCA  
5881 GGGCCGAGCC TACATGTGCG AATGATGCCC ATACTTGAGC CACCTAAGTT TGTTTTAGGG  
5941 CGACTGCCCT GCTGCGTAAC ATCGTTGCTG CTGCGTAACA TCGTTGCTGC TCCATAACAT  
6001 CAAACATCGA CCCACGGCGT AACGCGCTTG CTGCTTGGAT GCCCGAGGCA TAGACTGTAC-



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6061 AAAAAACAG TCATAACAAG CCATGAAAAC CGCCACTGCG CCGTTACCAC CGCTGCGTTC  
6121 GGTCAAGGTT CTGGACCAGT TGGGTGAGCG CATACGCTAC TTGCATTACA GTTTACGAAC  
6181 CGAACAGGCT TATGTCAACT GGGTTCGTGC CTTTCATCCGT TTCCACGGTG TCGGTCACCC  
6241 GGCAACCTTG GGCAGCAGCG AAGTCGAGGC ATTTCTGTCC TGGCTGGCGA ACGAGCGCAA  
6301 GGTTTCGGTC TCCACGCATC GTCAGGCATT GGCGGCCTTG CTGTTCTTCT ACGGCAAGGT  
6361 GCTGTGCACG GATCTGCCCT GGCTTCAGGA GATCGGAAGA CCTCGGCCGT CGCGGCGCTT  
6421 GCCGGTGGTG CTGACCCCGG ATGAAGTGGT TCGCATCCTC GGTTTTCTGG AAGGCGAGCA  
6481 TCGTTTGTTT GCCCAGGACT CTAGCTATAG TTCTAGTGGT TGGCTA

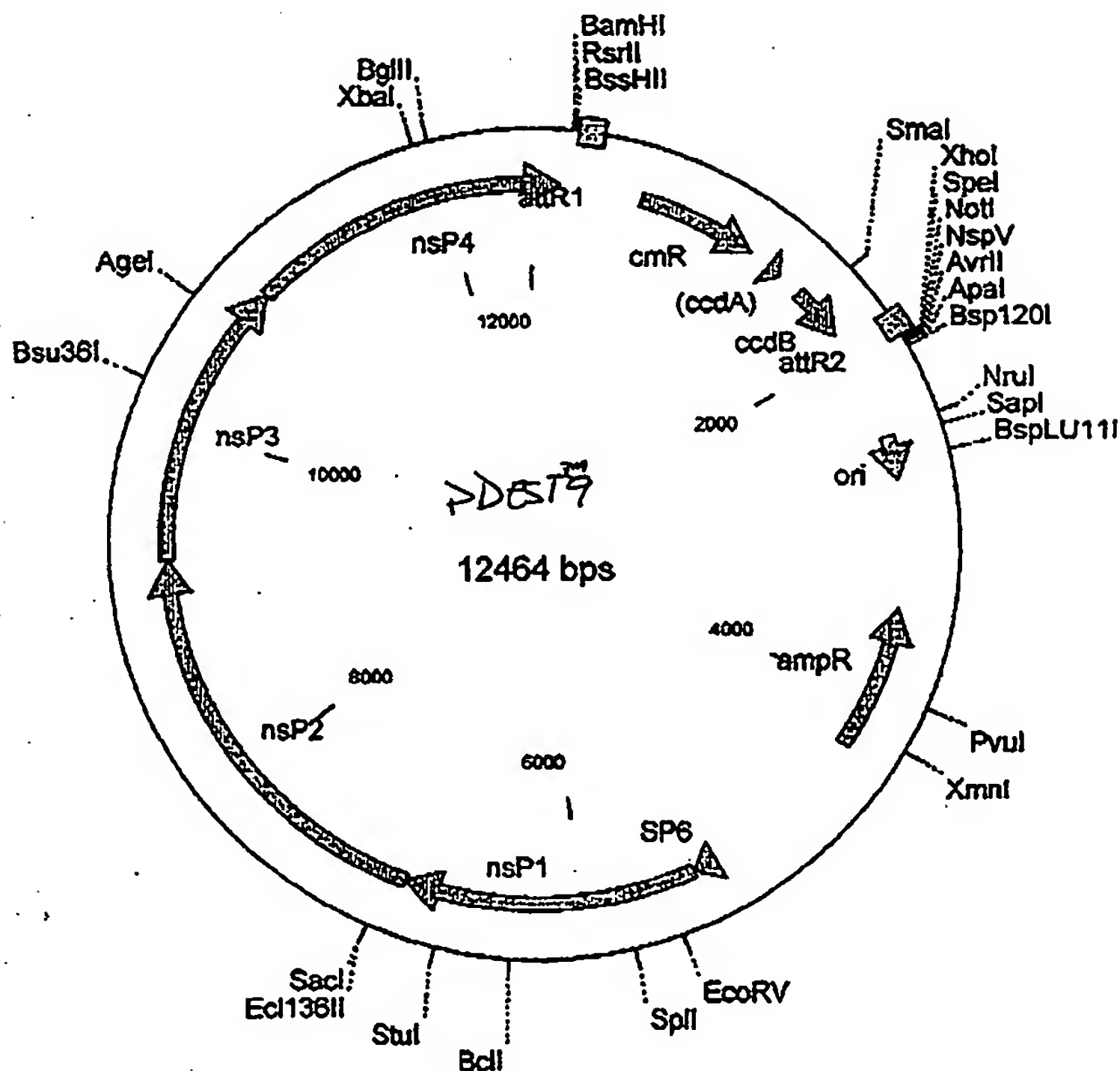
FIGURE 28A

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Figure 29A: pDEST9

Semliki Forest Virus vector

103 ttg gcg agg gac att aag gcg ttt aag aaa ttg aga gga cct gtt ata cac  
 aac cgc tcc ctg taa ttc cgc aaa ttc ttt aac tct cct gga caa tat gtg  
 265 promoter → 265 RNA Bam  
 154 ctc tac ggc ggt cct aga ttg gtc cgt taa tac aca gaa ttc tga ttg gat  
 gag atg ccg cca gga tct aac cac gca att atg tgt ctt aag act aac cta  
 Rsr II Irt att R1  
 205 ccc ggt ccg aag cgc gct ttc cca tca aca agt tgc tgc aac aac gct gaa  
ggg cca ggc ttc gcg cga aag ggt agt tgt tca aac atg ttt tta cga cta



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pDEST9 12464 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
355..232		attR1
605..1264		CmR
1384..1468		inactivated ccdA
1606..1911		ccdB
1952..2078		attR2
2532..2782		ori
3482..4282		ampR
5232..5365		SP6 promoter
5365..6965		nsP1:non-structural protein 1
6965..9265		nsP2:non-structural protein 2
9265..10865		nsP3:non-structural protein 3
10865..161		nsP4:non-structural protein 4
1 AGCAAGTGGT TCCGGACAGG CTTGGGGGCC GAACTGGAGG TGGCACTAAC ATCTAGGTAT		
61 GAGGTAGAGG GCTGCAAAAG TATCCTCATA GCCATGGCCA CCTTGGCGAG GGACATTAAG		
121 GCGTTTAAGA AATTGAGAGG ACCTGTTATA CACCTCTACG GCGGTCCTAG ATTGGTGCGT		
181 TAATACACAG AATTCTGATT GGATCCCGGT CCGAAGCGCG CTTTCCCATC ACAAGTTTGT		
241 ACAAAAAAGC TGAACGAGAA ACGTAAAATG ATATAAATAT CAATATATTA AATTAGATTT		
301 TGCATAAAAA ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC		
361 CGCTAAGTTG GCAGCATCAC CCGACGCACT TTGCGCCGAA TAAATACCTG TGACGGAAGA		
421 TCACTTCGCA GAATAAATAA ATCCTGGTGT CCCTGTTGAT ACCGGGAAGC CCTGGGCCAA		
481 CTTTTGGCGA AAATGAGACG TTGATCGGCA CGTAAGAGGT TCCAACTTTC ACCATAATGA		
541 AATAAGATCA CTACCGGGCG TATTTTTTGA GTTATCGAGA TTTTCAGGAG CTAAGGAAGC		
601 TAAAATGGAG AAAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA		
661 AGAACATTTT GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT		
721 GGATATTACG GCCTTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTTT ATCCGGCCTT		
781 TATTCACATT CTTGCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG CAATGAAAGA		
841 CGGTGAGCTG GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTTCC ATGAGCAAAC		
901 TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT		
961 ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT		
1021 TGAGAATATG TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAGTT TTGATTTAAA		
1081 CGTGGCCAAT ATGGACAAC TCTTCGCCCC CGTTTTCAAC ATGGGCAAAT ATTATACGCA		
1141 AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTAT CATGCCGTCT GTGATGGCTT		
1201 CCATGTCGGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC		
1261 GTAAAGATCT GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA		
1321 TTTTTCGCGT ATAAGAATAT ATACTGATAT GTATACCCGA AGTATGTCAA AAAGAGGTGT		
1381 GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA		
1441 TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA GCCCGTCGTC		
1501 TGCGTGCCGA ACGCTGGAAA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCCCGGTTTA		
1561 TTGAAATGAA CCGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT		
1621 TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT		
1681 GACACGCCCC GCGGACGGAT GGTGATCCCC CTGGCCAGTG CACGTCTGCT GTCAGATAAA		
1741 GTCTCCCGTG AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC		
1801 ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC		
1861 CGCGAAAATG ACATCAAAAA CGCCATTAACT CTGATGTTCT GGGGAATATA AATGTCAGGC		
1921 TCCCTTATAC ACAGCCAGTC TGCAGGTCGA CCATAGTGAC TGGATATGTT GTGTTTTACA		
1981 GTATTATGTA GTCTGTTTTT TATGCAAAAG TGCTAATTTA ATATATTGAT ATTTATATCA		
2041 TTTTACGTTT CTCGTTTACG TTTCTTGTA AAAGTGGTGA TGGGAACTCG AGTTCAGTAG		
2101 TCGATCCCCG GCGCGCTTTC GAACCTAGGC AAGCATGCGG GCCCAGTGGG TAATTAATTG		
2161 AATTACATCC CTACGCAAAC GTTTTACGGC CGCCGGTGGC GCCCGCGCCC GGCGGCCCGT		
2221 CCTTGGCCGT TGCAGGCCAC TCCGGTGGCT CCCGTCGTCC CCGACTTCCA GGCCAGCAG		
2281 ATGCAGCAAC TCATCAGCGC CGTAAATGCG CTGACAATGA GACAGAACGC AATTGCTCCT		
2341 GCTAGGAGCT TAATTCGACG AATAATTGGA TTTTATTTT ATTTTGCAAT TGGTTTTTAA		
2401 TATTTCCAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA-		

FIGURE 29B

2461 AAAAAAAAAA AAAAAAACTA GAAATCGCGA TTTCTAGTCT GCATTAATGA ATCGGCCAAC  
2521 GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC  
2581 TGCCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT  
2641 TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG  
2701 CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCTGACG  
2761 AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT  
2821 ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TCGCTCTCC TGTTCCGACC CTGCCGCTTA  
2881 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAA TGCTCGCGCT  
2941 GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG CACGAACCCC  
3001 CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACATATC TCTTGAGTCC AACCCGGTAA  
3061 GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG  
3121 TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG  
3181 TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT  
3241 GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTCGCAAG CAGCAGATTA  
3301 CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC  
3361 AGTGGAACGA AAATCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA  
3421 CCTAGATCCT TTAAATTAAT AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA  
3481 CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT  
3541 TTCGTTTCAT CATAGTTGCC TGAATCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT  
3601 TACCATCTGG CCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACC GCTCCAGATT  
3661 TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT  
3721 CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGAAGCTAG AGTAAGTAGT TCGCCAGTTA  
3781 ATAGTTTGGC CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG  
3841 GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCATGT  
3901 TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG  
3961 CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG  
4021 TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC  
4081 GCGGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA  
4141 CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTGGGGGCG AAAACTCTCA AGGATCTTAC  
4201 CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT  
4261 TTACTTTTAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG  
4321 GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTCAA TATTATTGAA  
4381 GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA  
4441 AACAAATAGG GGTTCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA  
4501 TTATTATCAT GACATTAACC TATAAAAATA GGCATATCAC GAGGCCCTTT CGTCTCGCGC  
4561 GTTTCGGTGA TGACGGTGAA AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT  
4621 CTGTCTAAGC GGATGCCGGG AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG  
4681 GGTGTCGGGG CTGGCTTAAC TATGCGGCAT CAGAGCAGAT TGTACTGAGA GTGCACCATA  
4741 TCGACGCTCT CCCTTATGCG ACTCCTGCAT TAGGAAGCAG CCCAGTACTA GGTGAGGCC  
4801 GTTGAGCACC GCCGCCGCAA GGAATGGTGC ATGCAAGGAG ATGGCGCCCA ACAGTCCCCC  
4861 GGCCACGGGG CCTGCCACCA TACCCACGCC GAAACAAGCG CTCATGAGCC CGAAGTGGCG  
4921 AGCCCGATCT TCCCCATCGG TGATGTCGGC GATATAGGCG CCAGCAACCG CACCTGTGGC  
4981 GCCGGTGATG CCGGCCACGA TGCCTCCGGC GTAGAGGATC TGGCTAGCGA TGACCTGCT  
5041 GATTGGTTCG CTGACCATTT CCGGGGTGCG GAACGGCGTT ACCAGAACT CAGAAGGTTT  
5101 GTCCAACCAA ACCGACTCTG ACGGCAGTTT ACGAGAGAGA TGATAGGGTC TGCTTCAGTA  
5161 AGCCAGATGC TACACAATTA GGCTTGTA CAATTGTCGTT AGAACGCGGC TACAATTAAT  
5221 ACATAACCTT ATGTATCATA CACATACGAT TTAGGTGACA CTATAGATGG CGGATGTGTG  
5281 ACATACACGA CGCCAAAAGA TTTTGTTC CA GCTCCTGCCA CCTCCGCTAC GCGAGAGATT  
5341 AACCACCCAC GATGGCCGCC AAAGTGCATG TTGATATTGA GGCTGACAGC CCATTCATCA  
5401 AGTCTTTGCA GAAGGCATTT CCGTCGTTTC AGGTGGAGTC ATTGCAGGTC ACACCAAATG  
5461 ACCATGCAAA TGCCAGAGCA TTTTCGACC TGGCTACCAA ATTGATCGAG CAGGAGACTG  
5521 ACAAAGACAC ACTCATCTTG GATATCGGCA GTGCGCCTTC CAGGAGAAATG ATGTCTACGC  
5581 ACAAATACCA CTGCGTATGC CCTATGCGCA GCGCAGAAGA CCCCAGAAAG CTCGATAGCT  
5641 ACGCAAAGAA ACTGGCAGCG GCCTCCGGGA AGGTGCTGGA TAGAGAGATC GCAGGAAAAA  
5701 TCACCGACCT GCAGACCGTC ATGGCTACGC CAGACGCTGA ATCTCCTACC TTTTGCCTGC  
5761 ATACAGACGT CACGTGTCGT ACGGCAGCCG AAGTGGCCGT ATACCAGGAC GTGTATGCTG  
5821 TACATGCACC AACATCGCTG TACCATCAGG CGATGAAAGG TGTCAGAACG GCGTATTGGA  
5881 TTGGGTTTGA CACCACCCCG TTTATGTTG ACGCGCTAGC AGGCGCGTAT CCAACCTACG-



5941 CCACAAACTG GGCCGACGAG CAGGTGTTAC AGGCCAGGAA CATAGGACTG TGTGCASCAT  
6001 CCTTGACTGA GGGAAAGACTC GGCAAACGTG CCATTCTCCG CAAGAAGCAA TTGAAACCTT  
6061 GCGACACAGT CATGTTCTCG GTAGGATCTA CATTGTACAC TGAGAGCAGA AAGCTACTGA  
6121 GGAGCTGGCA CTTACCCTCC GTATTCCACC TGAAAGGTAA ACAATCCTTT ACCTGTAGGT  
6181 GCGATACCAT CGTATCATGT GAAGGGTACG TAGTTAAGAA AATCACTATG TGCCCCCGCC  
6241 TGTACGGTAA AACGGTAGGG TACGCCGTGA CGTATCACGC GGAGGGATTG CTAGTGTGCA  
6301 AGACCACAGA CACTGTCAAA GGAGAAAGAG TCTCATTCCC TGTATGCACC TACGTCCCCT  
6361 CAACCATCTG TGATCAAATG ACTGGCATACT TAGCGACCGA CGTCACACCG GAGGACGCAC  
6421 AGAAGTTGTT AGTGGGATTG AATCAGAGGA TAGTTGTGAA CGGAAGAACA CAGCGAAACA  
6481 CTAACACGAT GAAGAACTAT CTGCTTCCGA TTGTGGCCGT CGCATTTAGC AAGTGGSCGA  
6541 GGGAATACAA GGCAGACCTT GATGATGAAA AACCTCTGGG TGTCCGAGAG AGGTCACCTA  
6601 CTTGCTGCTG CTTGTGGGCA TTAAAACGA GGAAGATGCA CACCATGTAC AAGAAACCAG  
6661 ACACCCAGAC AATAGTGAAG GTGCCCTTCA AGTTTAACTC GTTCGTCATC CCGAGCCTAT  
6721 GGTCTACAGG CCTCGCAATC CCAAGTCAGT CACGCATTAA GATGCTTTTG GCCAAGAAGA  
6781 CCAAGCGAGA GTTAATACCT GTTCTCGACG CGTCGTCAGC CAGGGATGCT GAACAAGAGG  
6841 AGAAGGAGAG GTTGGAGGCC GAGCTGACTA GAGAAGCCTT ACCACCCCTC GTCCCCATCG  
6901 CGCCGGCGGA GACGGGAGTC GTCGACGTCG ACGTTGAAGA ACTAGAGTAT CACGCAAGTG  
6961 CAGGGGTCGT GGAAACACCT CGCAGCGCGT TGAAAGTCAC CGCACAGCCG AACGACGTAC  
7021 TACTAGGAAA TTACGTAGTT CTGTCCCCGC AGACCGTGCT CAAGAGCTCC AAGTTGSCCC  
7081 CCGTGCACCC TCTAGCAGAG CAGGTGAAAA TAATAACACA TAACGGGAGG GCCGGCGGTT  
7141 ACCAGGTCGA CGGATATGAC GGCAGGGTCC TACTACCATG TGGATCGGCC ATTCCGGTCC  
7201 CTGAGTTTCA GGCTTTGAGC GAGAGCGCCA CTATGGTGTA CAACGAAAGG GAGTTCGTCA  
7261 ACAGGAACT ATACCATATT GCCGTTACG GACCCTCGCT GAACACCGAC GAGGAGAACT  
7321 ACGAGAAAGT CAGAGCTGAA AGAACTGACG CCGAGTACGT GTTCGACGTA GATAAAAAAT  
7381 GCTGCGTCAA GAGAGAGGAA GCGTCGGGTT TGGTGTGGT GGGAGAGCTA ACCAACCCCC  
7441 CGTTCCATGA ATTCGCCTAC GAAGGGCTGA AGATCAGGCC GTCGGCACCA TATAAGACTA  
7501 CAGTAGTAGG AGTCTTTGGG GTTCCGGGAT CAGGCAAGTC TGCTATTATT AAGAGCCTCG  
7561 TGACCAAACA CGATCTGGTC ACCAGCGGCA AGAAGGAGAA CTGCCAGGAA ATAGTTAAGC  
7621 ACGTGAAGAA GCACCGCGGG AAGGGGACAA GTAGGGAAAA CAGTGACTCC ATCCTGCTAA  
7681 ACGGGTGTG TCGTGCCGTG GACATCCTAT ATGTGGACGA GGCTTTCGCT TGCCATTCCG  
7741 GTACTCTGCT GGCCCTAATT GCTCTTGTTA AACCTCGGAG CAAAGTGGTG TTATGCAGAG  
7801 ACCCAAGCA ATGCGGATTC TTCAATATGA TGCAGCTTAA GGTGAACCTC AACCACAACA  
7861 TCTGCACTGA AGTATGTCAT AAAAGTATAT CCAGACGTTG CACGCGTCCA GTCACG3CCA  
7921 TCGTGTCTAC GTTGCACTAC GGAGGCAAGA TCGCACGAC CAACCCGTGC AACAAACCCA  
7981 TAATCATAGA CACCACAGGA CAGACCAAGC CCAAGCCAGG AGACATCGTG TTAACATGCT  
8041 TCCGAGGCTG GGCAAAGCAG CTGCAGTTGG ACTACCGTGG ACACGAAGTC ATGACAGCAG  
8101 CAGCATCTCA GGGCCTCACC CGCAAAGGGG TATACGCCGT AAGGCAGAAG GTGAAT3AAA  
8161 ATCCCTTGTA TGCCCCGTG TCGGAGCAGC TGAATGTACT GCTGACGCGC ACTGAG3ATA  
8221 GGCTGGTGTG GAAAACGCTG GCCGGCGATC CCTGGATTAA GGTCCCTATCA AACATTCCAC  
8281 AGGGTAACTT TACGGCCACA TTGGAAGAAT GGCAAGAAGA ACACGACAAA ATAATGAAGG  
8341 TGATTGAAGG ACCGGCTGCG CCTGTGGACG CGTTCAGAA CAAAGCGAAC GTGTGTTGGG  
8401 CGAAAAGCCT GGTGCCTGTC CTGGACACTG CCGGAATCAG ATTGACAGCA GAGGAGTGGG  
8461 GCACCATAAT TACAGCATTT AAGGAGGACA GAGCTTACTC TCCAGTGGTG GCCTTGAATG  
8521 AAATTTGCAC CAAGTACTAT GGAGTTGACC TGGACAGTGG CCTGTTTTCT GCCCCGAAGG  
8581 TGTCCCTGTA TTACGAGAAC AACCAGTGGG ATAACAGACC TGGTGAAGG ATGTAT3GAT  
8641 TCAATGCCGC AACAGCTGCC AGGCTGGAAG CTAGACATAC CTTCTGTAAG GGGCAGTGGC  
8701 ATACGGGCAA GCAGGCAGTT ATCGCAGAAA GAAAAATCCA ACCGCTTTCT GTGCTG3ACA  
8761 ATGTAATTCC TATCAACCGC AGGCTGCCGC ACGCCCTGGT GGCTGAGTAC AAGACGGTTA  
8821 AAGGCAGTAG GGTTGAGTGG CTGGTCAATA AAGTAAGAGG GTACCACGTC CTGCTGGTGA  
8881 GTGAGTACAA CCTGGCTTTG CCTCGACGCA GGGTCACTTG GTTGTACCCG CTGAATGTCA  
8941 CAGGCGCCGA TAGGTGCTAC GACCTAAGTT TAGGACTGCC GGCTGACGCC GGCAGGTTCC  
9001 ACTTGGTCTT TGTGAACATT CACACGGAAT TCAGAATCCA CCACTACCAG CAGTGTGTCC  
9061 ACCACGCCAT GAAGCTGCAG ATGCTTGGGG GAGATGCGCT ACGACTGCTA AAACCCGGCG  
9121 GCATCTTGAT GAGAGCTTAC GGATACGCCG ATAAAATCAG CGAAGCCGTT GTTTCCTCCT  
9181 TAAGCAGAAA GTTCTCGTCT GCAAGAGTGT TGCGCCCGGA TTGTGTCACC AGCAATACAG  
9241 AAGTGTCTT GCTGTTCTCC AACTTTGACA ACGGAAAGAG ACCCTCTACG CTACACCAGA  
9301 TGAATACCAA GCTGAGTGCC GTGTATGCCG GAGAAGCCAT GCACACGGCC GGGTGTGCAC  
9361 CATCCTACAG AGTTAAGAGA GCAGACATAG CCACGTGCAC AGAAGCGGCT GTGGTTAACG

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9421 CAGCTAACGC CCGTGGAAC TTAGGGGATG GCGTATGCAG GGCCGTGGCG AAGAAATGGC  
9481 CGTCAGCCTT TAAGGGAGCA GCAACACCAG TGGGCACAAT TAAAACAGTC ATGTGCGGCT  
9541 CGTACCCCGT CATCCACGCT GTAGCGCCTA ATTTCTCTGC CACGACTGAA GCGGAAGGGG  
9601 ACCGCGAATT GGCCGCTGTC TACCGGGCAG TGGCCGCCGA AGTAAACAGA CTGTCACTGA  
9661 GCAGCGTAGC CATCCCGCTG CTGTCCACAG GAGTGTTCAG CGGCGGAAGA GATAGGCTGC  
9721 AGCAATCCCT CAACCATCTA TTCACAGCAA TGGACGCCAC GGACGCTGAC GTGACCATCT  
9781 ACTGCAGAGA CAAAAGTTGG GAGAAGAAAA TCCAGGAAGC CATTGACATG AGGACGGCTG  
9841 TGGAGTTGCT CAATGATGAC GTGGAGCTGA CCACAGACTT GGTGAGAGTG CACCCGGACA  
9901 GCAGCCTGGT GGGTCGTAAG GGCTACAGTA CCACTGACGG GTCGCTGTAC TCGTACTTTG  
9961 AAGGTACGAA ATTCAACCAG GCTGCTATTG ATATGGCAGA GATACTGACG TTGTGGCCCA  
10021 GACTGCAAGA GGCAAACGAA CAGATATGCC TATACGCGCT GGGCGAAACA ATGGACAACA  
10081 TCAGATCCAA ATGTCCGGTG AACGATTCCG ATTCATCAAC ACCTCCCAGG ACAGTGCCCT  
10141 GCCTGTGCCG CTACGCAATG ACAGCAGAAC GGATCGCCCG CCTTAGGTCA CACCAAGTTA  
10201 AAAGCATGGT GGTTTGCTCA TCTTTTCCCC TCCCGAAATA CCATGTAGAT GGGGTGCAGA  
10261 AGGTAAAGTG CGAGAAGGTT CTCCTGTTCTG ACCCGACGGT ACCTTCAGTG GTTAGTCCGC  
10321 GGAAGTATGC CGCATCTACG ACGGACCACT CAGATCGGTC GTTACGAGGG TTTGACTTGG  
10381 ACTGGACCAC CGACTCGTCT TCCACTGCCA GCGATACCAT GTCGCTACCC AGTTTGCACT  
10441 CGTGTGACAT CGACTCGATC TACGAGCCAA TGGCTCCCAT AGTAGTGACG GCTGACGTAC  
10501 ACCCTGAACC CGCAGGCATC GCGGACCTGG CCGCAGATGT GCACCCTGAA CCCGCAGACC  
10561 ATGTGGACCT GGAGAACCCG ATTCTCCAC CGCGCCCGAA GAGAGCTGCA TACCTTGCCCT  
10621 CCCGCGCGGC GGAGCGACCG GTGCCGGCGC CGAGAAAGCC GACGCCTGCC CCAAGGACTG  
10681 CGTTTAGGAA CAAGCTGCCT TTGACGTTCTG GCGACTTTGA CGAGCACGAG GTCGATGCGT  
10741 TGGCCTCCGG GATTACTTTC GGAGACTTCG ACGACGTCCT GCGACTAGGC CGCGCGGGTG  
10801 CATATATTTT CTCCTCGGAC ACTGGCAGCG GACATTTACA AAAAAATCC GTTAGGCAGC  
10861 ACAATCTCCA GTGCGCACAA CTGGATGCGG TCCAGGAGGA GAAAATGTAC CCGCCAAAAT  
10921 TGGATACTGA GAGGGAGAAG CTGTTGCTGC TGAAAATGCA GATGCACCCA TCGGAGGCTA  
10981 ATAAGAGTCG ATACCAGTCT CGCAAAGTGG AGAACATGAA AGCCACGGTG GTGGACAGGC  
11041 TCACATCGGG GGCCAGATTG TACACGGGAG CCGACGTAGG CCGCATACCA ACATACGCGG  
11101 TTCGGTACCC CCGCCCCGTG TACTCCCCCTA CCGTGATCGA AAGATTCTCA AGCCCCGATG  
11161 TAGCAATCGC AGCGTGCAAC GAATACCTAT CCAGAAATTA CCCAACAGTG GCGTCGTACC  
11221 AGATAACAGA TGAATACGAC GCATACTTGG ACATGGTTGA CGGGTCGGAT AGTTGCTTGG  
11281 ACAGAGCGAC ATTCTGCCCC GCGAAGCTCC GGTGCTACCC GAAACATCAT GCGTACCACC  
11341 AGCCGACTGT ACGCAGTGCC GTCCCGTCAC CTTTTCAGAA CACACTACAG AACGTGCTAG  
11401 CGGCTGCCAC CAAGAGAAAC TGCAACGTCA CGCAAATGCG AGAACTACCC ACCATGGACT  
11461 CGGCAGTGTT CAACGTGGAG TGCTTCAAGC GCTATGCCTG CTCCGGAGAA TATTGGGAAG  
11521 AATATGCTAA ACAACCTATC CGGATAACCA CTGAGAACAT CACTACCTAT GTGACCAAAT  
11581 TGAAAGGCCG GAAAGCTGCT GCCTTGTTCTG CTAAGACCCA CAACTTGGTT CCGCTGCAGG  
11641 AGGTTCCCAT GGACAGATTC ACGGTCGACA TGAAACGAGA TGTCAAAGTC ACTCCAGGGA  
11701 CGAAACACAC AGAGGAAAGA CCCAAAGTCC AGGTAATTCA AGCAGCGGAG CCATTGGCGA  
11761 CCGCTTACCT GTGCGGCATC CACAGGGAAT TAGTAAGGAG ACTAAATGCT GTGTTACGCC  
11821 CTAACGTGCA CACATTGTTT GATATGTCGG CCGAAGACTT TGACGCGATC ATCGCCTCTC  
11881 ACTTCCACCC AGGAGACCCG GTTCTAGAGA CCGACATTGC ATCATTGAC AAAAGCCAGG  
11941 ACGACTCCTT GGCTCTTACA GGTTTAATGA TCCTCGAAGA TCTAGGGGTG GATCAGTACC  
12001 TGCTGGACTT GATCGAGGCA GCCTTTGGGG AAATATCCAG CTGTCACCTA CCAACTGGCA  
12061 CGCGCTTCAA GTTCGGAGCT ATGATGAAAT CGGGCATGTT TCTGACTTTG TTTATTAACA  
12121 CTGTTTTGAA CATCACCATA GCAAGCAGGG TACTGGAGCA GAGACTCACT GACTCCGCCT  
12181 GTGCGGCCTT CATCGGCGAC GACAACATCG TTCACGGAGT GATCTCCGAC AAGCTGATGG  
12241 CGGAGAGGTG CGCGTCGTGG GTCAACATGG AGGTGAAGAT CATTGACGCT GTCATGGGCG  
12301 AAAAACCCCC ATATTTTTGT GGGGGATTCA TAGTTTTTGA CAGCGTCACA CAGACCGCCT  
12361 GCCGTGTTTC AGACCCACTT AAGCGCCTGT TCAAGTTGGG TAAGCCGCTA ACAGCTGAAG  
12421 ACAAGCAGGA CGAAGACAGG CGACGAGCAC TGAGTGACGA GGTT

FIGURE 29E



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**Figure 30A: pDEST10 Polyhedron Promoter with N-His6, Baculovirus Transfer Plasmid**

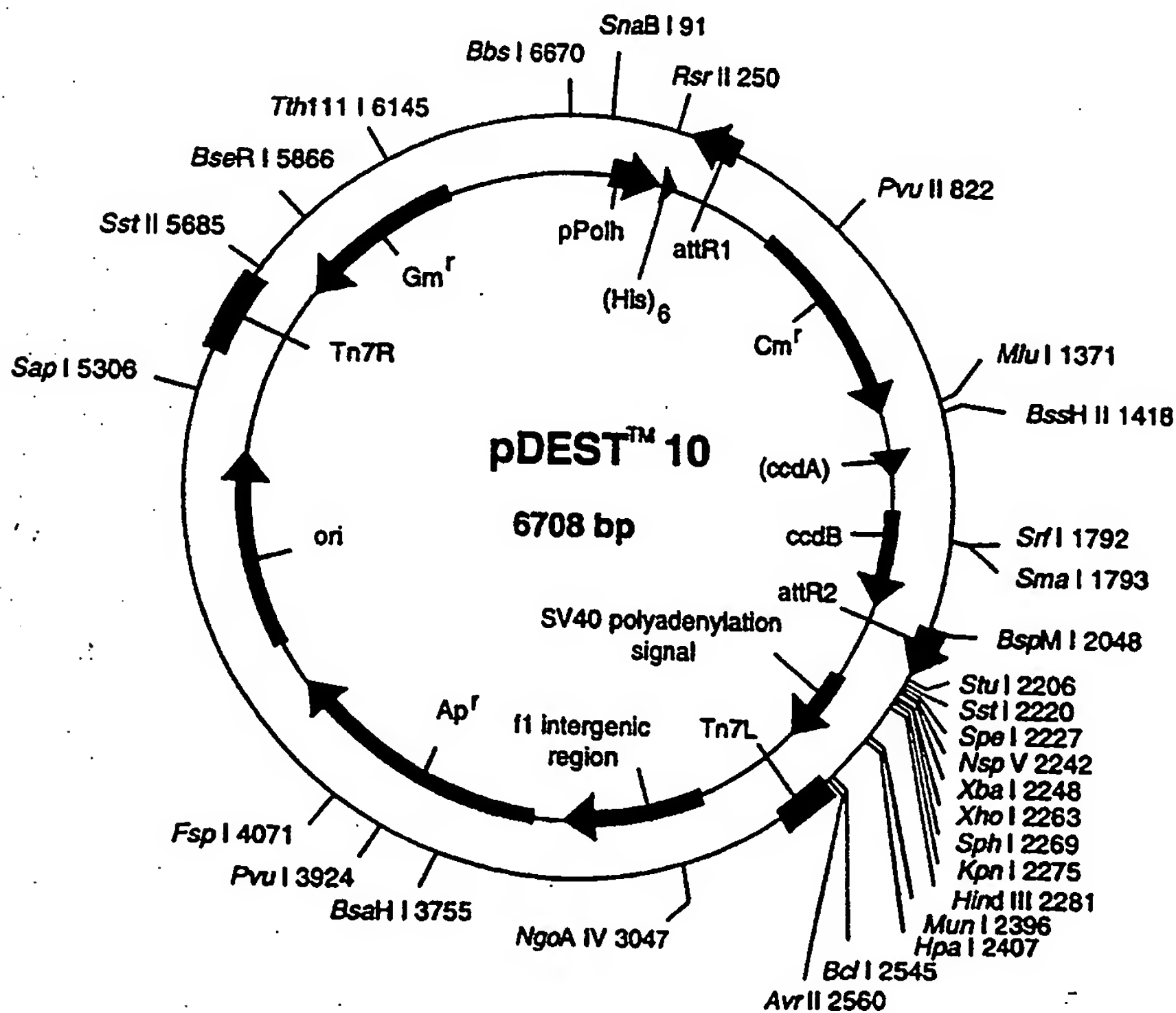
154 *mRat from polyhedrin promoter*  
 aaa taa gta ttt tac tgt ttt cgt aac agt ttt gta ata aaa aaa cct ata  
 ttt att cat aaa atg aca aaa gca ttg tca aaa cat tat ttt ttt gga tat

205  
 aat att ccg gat tat tca tac cgt ccc acc atc ggg cgc gga tct cgg tcc  
 tta taa ggc cta ata agt atg gca ggg tgg tag ccc gcg cct aga gcc agg

256  
 gaa acc <sup>Met</sup> atg tgg tac <sup>Ser</sup> tac <sup>Tyr</sup> tac <sup>Tyr</sup> cat <sup>His</sup> cac <sup>His</sup> cat <sup>His</sup> cac <sup>His</sup> cat <sup>His</sup> cat <sup>Asp</sup> gat <sup>Tyr</sup> tac <sup>Asp</sup> gat <sup>Ile</sup> atc <sup>Pro</sup> cca  
 ctt tgg tac agc atg atg gta gtg gta gtg gta gtg cta atg cta tag ggt

307  
 Tgt acc <sup>Glu</sup> gaa <sup>Asn</sup> aac ctg <sup>Leu</sup> tat <sup>Tyr</sup> ttt <sup>Phe</sup> cag <sup>Gln</sup> ggc <sup>Gly</sup> atc <sup>Ile</sup> ttt <sup>Thr</sup> aca <sup>Ser</sup> agt <sup>Leu</sup> tgg <sup>Tyr</sup> tac <sup>Lys</sup> aac <sup>Lys</sup> gaa gct  
 tgc tgg ctt ttg gac ata aaa gtc ccg tag tgt tca aac atg ttt ttc oga

*TEV protease*  
 attR1 Int



## pDEST10 6708 bp

Location (Base Nos.)		Gene Encoded
23..152		Ppolh
461..337		attR1
711..1370		CmR
1490..1574		inactivated ccdA
1712..2017		ccdB
2058..2182		attR2
3394..4369		ampR
4510..5164		ori
5658..62		genR

1	CCCCGGATGA	AGTGGTTCGC	ATCCTCGGTT	TTCTGGAAGG	CGAGCATCGT	TTGTTTCGCCC
61	AGGACTCTAG	CTATAGTTCT	AGTGGTTGGC	TACGTATACT	CCGGAATATT	AATAGATCAT
121	GGAGATAATT	AAAATGATAA	CCATCTCGCA	AATAAATAAG	TATTTTACTG	TTTTTCGTAAC
181	AGTTTTGTAA	TAAAAAACCC	TATAAATATT	CCGGATTATT	CATACCGTCC	CACCATCGGG
241	CGCGGATCTC	GGTCCGAAAC	CATGTCGTAC	TACCATCACC	ATCACCATCA	CGATTACGAT
301	ATCCCAACGA	CCGAAAACCT	GTATTTTCAG	GGCATCACAA	GTTTGTACAA	AAAAGCTGAA
361	CGAGAAACGT	AAAATGATAT	AAATATCAAT	ATATTAAATT	AGATTTTGCA	TAAAAACAG
421	ACTACATAAT	ACTGTAAAAC	ACAACATATC	CAGTCACTAT	GGCGGCCGCT	AAGTTGGCAG
481	CATCACCCGA	CGCACTTTGC	GCCGAATAAA	TACCTGTGAC	GGAAGATCAC	TTCGCAGAAT
541	AAATAAATCC	TGGTGTCCCT	GTTGATACCG	GGAAGCCCTG	GGCCAACTTT	TGGCGAAAAT
601	GAGACGTTGA	TCGGCACGTA	AGAGGTTCCA	ACTTTCACCA	TAATGAAATA	AGATCACTAC
661	CGGGCGTATT	TTTTGAGTTA	TCGAGATTTT	CAGGAGCTAA	GGAAGCTAAA	ATGGAGAAAA
721	AAATCACTGG	ATATACCACC	GTTGATATAT	CCCAATGGCA	TCGTAAAGAA	CATTTTGAGG
781	CATTTTCAGTC	AGTTGCTCAA	TGTACCTATA	ACCAGACCGT	TCAGCTGGAT	ATTACGGCCT
841	TTTTAAAGAC	CGTAAAGAAA	AATAAGCACA	AGTTTTATCC	GGCCTTTATT	CACATTCTTG
901	CCCGCCTGAT	GAATGCTCAT	CCGGAATTCC	GTATGGCAAT	GAAAGACGGT	GAGCTGGTGA
961	TATGGGATAG	TGTTCAACCCT	TGTTACACCG	TTTTCCATGA	GCAAACCTGAA	ACGTTTTTCAT
1021	CGCTCTGGAG	TGAATACCAC	GACGATTTCC	GGCAGTTTCT	ACACATATAT	TCGCAAGATG
1081	TGGCGTGTTA	CGGTGAAAAC	CTGGCCTATT	TCCCTAAAGG	GTTTATTGAG	AATATGTTTT
1141	TCGTCTCAGC	CAATCCCTGG	GTGAGTTTCA	CCAGTTTTGA	TTTAAACGTG	GCCAATATGG
1201	ACAACCTCTT	CGCCCCCGTT	TTCACCATGG	GCAAATATTA	TACGCAAGGC	GACAAGGTGC
1261	TGATGCCGCT	GGCGATTCAG	GTTTCATCATG	CCGTCTGTGA	TGGCTTCCAT	GTCGGCAGAA
1321	TGCTTAATGA	ATTACAACAG	TACTGCGATG	AGTGGCAGGG	CGGGGCGTAA	ACGCGTGGAT
1381	CCGGCTTACT	AAAAGCCAGA	TAACAGTATG	CGTATTTGCG	CGCTGATTTT	TGCGGTATAA
1441	GAATATATAC	TGATATGTAT	ACCCGAAGTA	TGTCAAAAAG	AGGTGTGCTA	TGAAGCAGCG
1501	TATTACAGTG	ACAGTTGACA	GCGACAGCTA	TCAGTTGCTC	AAGGCATATA	TGATGTCAAT
1561	ATCTCCGGTC	TGGTAAGCAC	AACCATGCAG	AATGAAGCCC	GTCGTCTGCG	TGCCGAACGC
1621	TGGAAAGCGG	AAAATCAGGA	AGGGATGGCT	GAGGTCGCCC	GGTTTATTGA	AATGAACGGC
1681	TCTTTTGCTG	ACGAGAACAG	GGACTGGTGA	AATGCAGTTT	AAGGTTTACA	CCTATAAAAG
1741	AGAGAGCCGT	TATCGTCTGT	TTGTGGATGT	ACAGAGTGAT	ATTATTGACA	CGCCCGGGCG
1801	ACGGATGGTG	ATCCCCCTGG	CCAGTGCACG	TCTGCTGTCA	GATAAAGTCT	CCCGTGAACT
1861	TTACCCGGTG	GTGCATATCG	GGGATGAAAG	CTGGCGCATG	ATGACCACCG	ATATGGCCAG
1921	TGTGCCGGTC	TCCGTTATCG	GGGAAGAAGT	GGCTGATCTC	AGCCACCGCG	AAAATGACAT
1981	CAAAAACGCC	ATTAACCTGA	TGTTCTGGGG	AATATAAATG	TCAGGCTCCC	TTATACACAG
2041	CCAGTCTGCA	GGTCGACCAT	AGTGACTGGA	TATGTTGTGT	TTTACAGTAT	TATGTAGTCT
2101	GTTTTTTATG	CAAAATCTAA	TTTAATATAT	TGATATTTAT	ATCATTTTAC	GTTTCTCGTT
2161	CAGCTTTCTT	GTACAAAGTG	GTGATGCCAT	GGATCCGGAA	TTCAAAGGCC	TACGTCGACG
2221	AGCTCAACTA	GTGCGGCCGC	TTTCGAATCT	AGAGCCTGCA	GTCTCGAGGC	ATGCGGTACC
2281	AAGCTTGTCG	AGAAGTACTA	GAGGATCATA	ATCAGCCATA	CCACATTTGT	AGAGGTTTTA
2341	CTTGCTTTAA	AAAACCTCCC	ACACCTCCCC	CTGAACCTGA	AACATAAAAT	GAATGCAATT
2401	GTTGTTGTTA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	TAGCATCACA
2461	AATTTTCAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTC	CAAACCTCATC
2521	AATGTATCTT	ATCATGTCTG	GATCTGATCA	CTGCTTGAGC	CTAGGAGATC	CGAACCAGAT
2581	AAGTGAAATC	TAGTTCCAAA	CTATTTTGTC	ATTTTTAATT	TTCGTATTAG	CTTACGACGC

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2641 TACACCCAGT TCCCATCTAT TTTGTCACTC TTCCCTAAAT AATCCTTAAA AACTCCATTT  
2701 CCACCCCTCC CAGTTCCCAA CTATTTTGTC CGCCACAGC GGGGCATTTT TCTTCCTGTT  
2761 ATGTTTTTAA TCAAACATCC TGCCAACTCC ATGTGACAAA CCGTCATCTT CGGCTACTTT  
2821 TTCTCTGTCA CAGAATGAAA ATTTTCTGT CATCTCTTCG TTATTAATGT TTGTAATTGA  
2881 CTGAATATCA ACGCTTATTT GCAGCCTGAA TGGCGAATGG GACGCGCCCT GTAGCGGCGC  
2941 ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT  
3001 AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG GCTTTCCCGG  
3061 TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA  
3121 CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT  
3181 TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAAACTGG  
3241 AACAACACTC AACCCTATCT CGGTCTATTC TTTTGATTGA TAAGGGATTT TGCCGATTTT  
3301 GGCCTATTGG TTAATAAATG AGCTGATTTA AAAAAAATT AACGCGAATT TTAACAAAAT  
3361 ATTAACGTTT ACAATTTTCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTGG  
3421 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT  
3481 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT  
3541 TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT  
3601 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTAC ATCGAACTGG ATCTCAACAG  
3661 CGGTAAGATC CTTGAGAGTT TTCGCCCGA AGAACGTTT CCAATGATGA GCACTTTTAA  
3721 AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTG  
3781 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT  
3841 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC  
3901 TGCGGCCAAC TTAATTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA  
3961 CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT  
4021 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TCGCGAAACT  
4081 ATTAAGTGGC GAAGTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC  
4141 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA  
4201 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CCGTATCATT GCAGCACTGG GGCCAGATGG  
4261 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG  
4321 AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA  
4381 AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA  
4441 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCGTTCCA  
4501 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG  
4561 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA  
4621 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA  
4681 TACTGTCCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC  
4741 TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG  
4801 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC  
4861 GGGGGGTTTCG TGCACACAGC CCAGCTTGGG GCGAACGACC TACACCGAAC TGAGATACCT  
4921 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC  
4981 GGTAAAGCGG AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG  
5041 GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG  
5101 CTCGTACAGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTTCT  
5161 GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG TTATCCCCTG ATTCTGTGGA  
5221 TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG  
5281 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG CCGTATTTTC TCCTTACGCA  
5341 TCTGTGCGGT ATTTACACAC GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG  
5401 AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAAACTGAA  
5461 CAAAATAGAT CTAAACTATG ACAATAAAGT CTAAACTAG ACAGAATAGT TGTAAGTGA  
5521 AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTTATGGCT AAAGCAAAC  
5581 CTTCAATTTT TGAAGTGCAA ATTGCCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG  
5641 GTAAAGACTA TATTGCGGGC GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC  
5701 GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGCATCAC  
5761 TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC  
5821 TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG  
5881 CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAGACT GCGAGATCAT AGATATAGAT  
5941 CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC  
6001 TTCTTGGTGC AAGGCAGCAA GCGCGATGAA TGTCTTACTA CGGAGCAAGT TCCCGAGGTA  
6061 ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG-

FIGURE 30C

6121 ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG TCGAATGAT  
6181 GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT  
6241 GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG  
6301 CTTGCTGCTT GGATGCCCGA GGCATAGACT GTACAAAAAA ACAGTCATAA CAAGCCATGA  
6361 AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA GGTTCCTGGAC CAGTTGCGTG  
6421 AGCGCATACG CTACTTGCAT TACAGTTTAC GAACCGAACA GGCTTATGTC AACTGGGTTC  
6481 GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC AGCGAAGTCG  
6541 AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG CATCGTCAGG  
6601 CATTGGCGGC CTTGCTGTTC TTCTACGGCA AGGTGCTGTG CACGGATCTG CCCTGGCTTC  
6661 AGGAGATCGG AAGACCTCGG CCGTCGCGC GCTTGCCGGT GGTGCTGA

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Figure 31A:

pDEST11

Tet-regulated eukaryotic  
expression

358 tag tga acc ggc aga tgg cct gga gac gcc atc cac gct gtt ttg acc tcc  
 atc act tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg

409 ata gaa gac acc ggg acc gat cca gcc tcc gcg gcc ccg aat tgg agc tgg  
 tat ctt ctg tgg ccc tgg cta ggt cgg agc cgc cgg ggc tta agc tgg agc

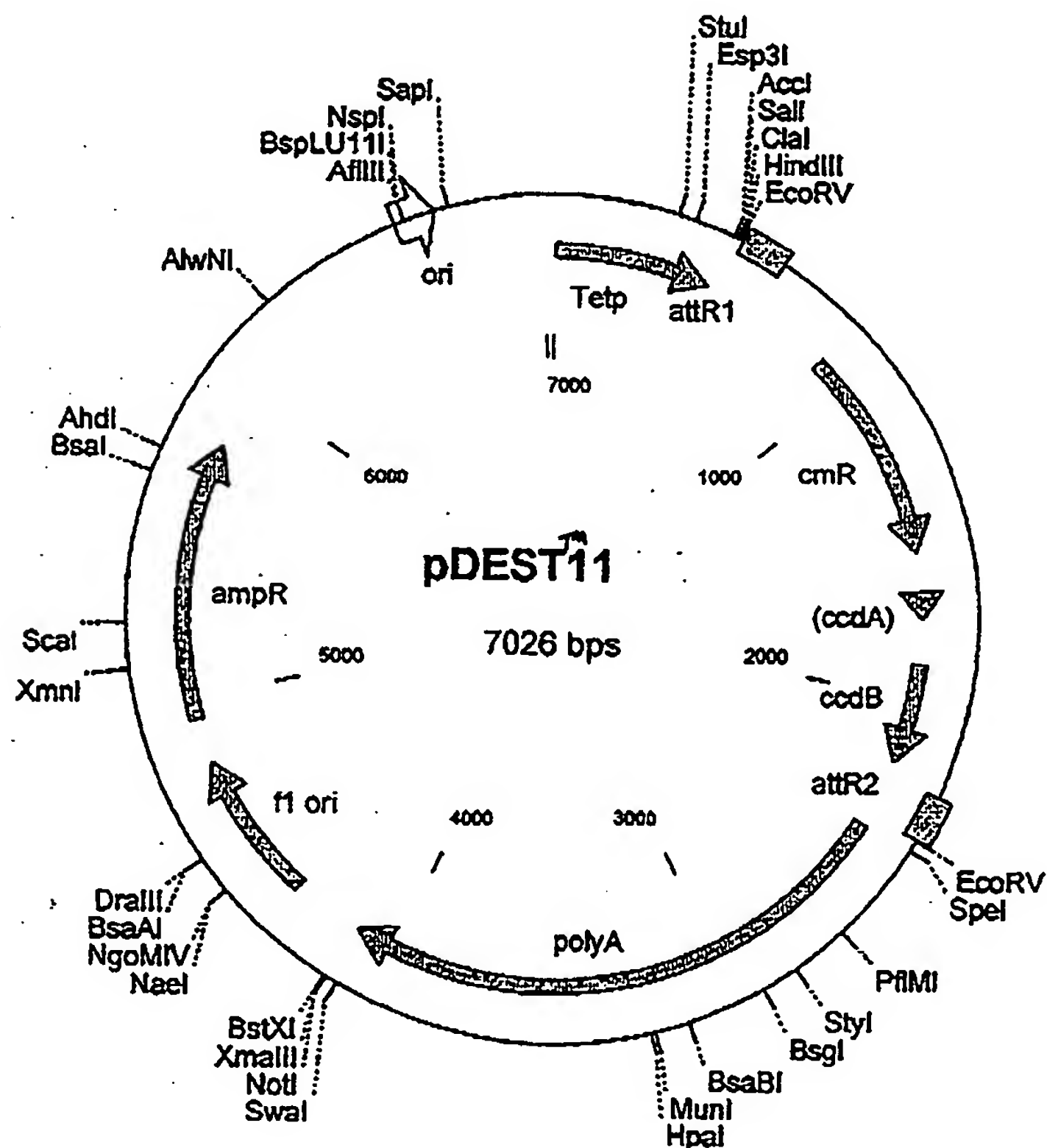
460 gta ccc ggg gat cct cta gag tgg agg tgg acg gta tgg ata agc ttg aga  
 cat ggg ccc cta gga gat ctc agc tcc agc tgc cat agc tat tgg acc tat

511 tca aca agt tgg ~~aga aac gct gaa cga gaa acg taa aat gat ata aat~~  
 agt ~~tgt tca aac atg ttt tct cga ctt gct cta tgc att tta cta tat tta~~

*mRNA from CMV promoter (controlled by tetracycline)*

*SalI* *ClaI* *Hind3* *EcoRV*

*I<sub>tr</sub>* *attR1*





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## pDEST11 7026 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
4..479		Tetp ((Tet operator) 7 and min hCMV promoter)
638..514		attR1
888..1547		CmR
1667..1751		inactivated ccdA
1889..2194		ccdB
2235..2359		attR2
2402..4132		polyA
4347..4803		f1 ori
4940..5797		ampR

1	CGAGTTTACC	ACTCCCTATC	AGTGATAGAG	AAAAGTGAAA	GTCGAGTTTA	CCACTCCCTA
61	TCAGTGATAG	AGAAAAGTGA	AAGTCGAGTT	TACCACTCCC	TATCAGTGAT	AGAGAAAAGT
121	GAAAGTCGAG	TTTACCACTC	CCTATCAGTG	ATAGAGAAAA	GTGAAAGTCG	AGTTTACCAC
181	TCCCTATCAG	TGATAGAGAA	AAGTGAAAGT	CGAGTTTACC	ACTCCCTATC	AGTGATAGAG
241	AAAAGTGAAA	GTCGAGTTTA	CCACTCCCTA	TCAGTGATAG	AGAAAAGTGA	AAGTCGAGCT
301	CGGTACCCGG	GTCGAGTAGG	CGTGTACGGT	GGGAGGCCCTA	TATAAGCAGA	GCTCGTTTAG
361	TGAACCGTCA	GATCGCCTGG	AGACGCCATC	CACGCTGTTT	TGACCTCCAT	AGAAGACACC
421	GGGACCGATC	CAGCCTCCGC	GGCCCCGAAT	TCGAGCTCGG	TACCCGGGGA	TCCTCTAGAG
481	TCGAGGTCGA	CGGTATCGAT	AAGCTTGATA	TCAACAAGTT	TGTACAAAAA	AGCTGAACGA
541	GAAACGTAAA	ATGATATAAA	TATCAATATA	TTAAATTAGA	TTTTGCATAA	AAAACAGACT
601	ACATAATACT	GTAAACACA	ACATATCCAG	TCATATGGC	GGCCGCTAAG	TTGGCAGCAT
661	CACCCGACGC	ACTTTGCGCC	GAATAAATAC	CTGTGACGGA	AGATCACTTC	GCAGAATAAA
721	TAAATCCTGG	TGTCCCTGTT	GATACCGGGA	AGCCCTGGGC	CAACTTTTGG	CGAAAATGAG
781	ACGTTGATCG	GCACGTAAGA	GGTTCCAAC	TTCACCATAA	TGAAATAAGA	TCACTACCGG
841	GCGTATTTTT	TGAGTTATCG	AGATTTTCAG	GAGCTAAGGA	AGCTAAAATG	GAGAAAAAAA
901	TCCTGGATA	TACCACCGTT	GATATATCCC	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT
961	TTCAGTCAGT	TGCTCAATGT	ACCTATAACC	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT
1021	TAAAGACCGT	AAAGAAAAAT	AAGCACAAGT	TTTATCCGGC	CTTTATTAC	ATTCTTGCCC
1081	GCCTGATGAA	TGCTCATCCG	GAATTCGGTA	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT
1141	GGGATAGTGT	TCACCCTTGT	TACACCGTTT	TCCATGAGCA	AACTGAAACG	TTTTCATCGC
1201	TCTGGAGTGA	ATACCACGAC	GATTTCCGGC	AGTTTCTACA	CATATATTCT	CAAGATGTGG
1261	CGTGTACGG	TGAAAACCTG	GCCTATTTCC	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG
1321	TCTCAGCCAA	TCCCTGGGTG	AGTTTCACCA	GTTTTGATTT	AAACGTGGCC	AATATGGACA
1381	ACTTCTTCGC	CCCCGTTTTT	ACCATGGGCA	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA
1441	TGCCGCTGGC	GATTCAGGTT	CATCATGCCG	TCTGTGATGG	CTTCCATGTC	GGCAGAAATG
1501	TTAATGAATT	ACAACAGTAC	TGCGATGAGT	GGCAGGGCGG	GGCGTAAAGA	TCTGGATCCG
1561	GCTTACTAAA	AGCCAGATAA	CAGTATGCGT	ATTTGCGCGC	TGATTTTTGC	GGTATAAGAA
1621	TATATACTGA	TATGTATACC	CGAAGTATGT	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT
1681	TACAGTGACA	GTTGACAGCG	ACAGCTATCA	GTTGCTCAAG	GCATATATGA	TGTCAATATC
1741	TCCGGTCTGG	TAAGCACAAC	CATGCAGAAT	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG
1801	AAAGCGGAAA	ATCAGGAAGG	GATGGCTGAG	GTCGCCCGGT	TTATTGAAAT	GAACGGCTCT
1861	TTTGCTGACG	AGAACAGGGA	CTGGTGAAAT	GCAGTTTAAG	GTTTACACCT	ATAAAAGAGA
1921	GAGCCGTTAT	CGTCTGTTTG	TGGATGTACA	GAGTGATATT	ATTGACACGC	CCGGGCGACG
1981	GATGGTGATC	CCCCTGGCCA	GTGCACGTCT	GCTGTGAGAT	AAAGTCTCCC	GTGAACTTTA
2041	CCCGGTGGTG	CATATCGGGG	ATGAAAGCTG	GCGCATGATG	ACCACCGATA	TGGCCAGTGT
2101	GCCGGTCTCC	GTTATCGGGG	AAGAAGTGGC	TGATCTCAGC	CACCGCGAAA	ATGACATCAA
2161	AAACGCCATT	AACCTGATGT	TCTGGGGAAT	ATAAATGTCA	GGCTCCCTTA	TACACAGCCA
2221	GTCTGCAGGT	CGACCATAGT	GACTGGATAT	GTTGTGTTTT	ACAGTATTAT	GTAGTCTGTT
2281	TTTTATGCAA	AATCTAATTT	AATATATTGA	TATTTATATC	ATTTTACGTT	TCTCGTTTCA
2341	CTTTCTTGTA	CAAAGTGGTT	GATATCGAAT	TCCTGCAGCC	CGGGGGATCC	ACTAGTTCTA
2401	GAGCACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAATTTTTTT	AAGGCAGTTA	TTGGTGCCCT
2461	TAAACGCCTG	GTGCTACGCC	TGAATAAGTG	ATAATAAGCG	GATGAATGGC	AGAAATTCGC
2521	CGGATCTTTG	TGAAGGAACC	TTACTTCTGT	GGTGTGACAT	AATTGGACAA	ACTACCTACA-

FIGURE 31B



2581 GAGATTTAAA GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTACTG  
2641 ATTCTAATTG TTTGTGTATT TTAGATTCCA ACCTATGGAA CTGATGAATG GGAGCAGTGG  
2701 TGGAATGCCT TTAATGAGGA AAACCTGTTT TGCTCAGAAG AAATGCCATC TAGTGATGAT  
2761 GAGGCTACTG CTGACTCTCA ACATTCTACT CCTCCAAAA AGAAGAGAAA GGTAGAAGAC  
2821 CCAAGGACT TTCCTTCAGA ATTGCTAAGT TTTTGTAGTC ATGCTGTGTT TAGTAATAGA  
2881 ACTCTTGCTT GCTTTGCTAT TTACACCACA AAGGAAAAAG CTGCACTGCT ATACAAGAAA  
2941 ATTATGGAAA AATATTCTGT AACCTTTATA AGTAGGCATA ACAGTTATAA TCATAACATA  
3001 CTGTTTTTTC TTACTCCACA CAGGCATAGA GTGTCTGCTA TTAATAACTA TGCTCAAAAA  
3061 TTGTGTACCT TTAGCTTTTT AATTTGTAAA GGGGTAAATA AGGAATATTT GATGTATAGT  
3121 GCCTTGACTA GAGATCATAA TCAGCCATAC CACATTTGTA GAGGTTTTAC TTGCTTTAAA  
3181 AAACCTCCCA CACCTCCCC TGAACCTGAA ACATAAAATG AATGCAATTG TTGTGTGTTAA  
3241 CTGTGTTTAT GCAGCTTATA ATGGTTACAA ATAAAGCAAT AGCATCACAA ATTTACACAA  
3301 TAAAGCATTT TTTTCACTGC ATTCTAGTTG TGGTTTGTCC AAACATCATCA ATGTATCTTA  
3361 TCATGTCTGG ATCCCCAGGA AGCTCCTCTG TGTCTCATA AACCTAACC TCCTCTACTT  
3421 GAGAGGACAT TCCAATCATA GGCTGCCCAT CCACCCTCTG TGTCTCTCTG TTAATTAGGT  
3481 CACTTAACAA AAAGGAAATT GGGTAGGGT TTTTCACAGA CCGCTTTCTA AGGGTAATTT  
3541 TAAATATCTT GGAAGTCCC TTCCACTGCT GTGTTCAGA AGTGTGTTGTA AACAGCCAC  
3601 AAATGTCAAC AGCAGAAACA TACAAGCTGT CAGCTTTGCA CAAGGGCCCA ACACCCTGCT  
3661 CATCAAGAAG CACTGTGGTT GCTGTGTTAG TAATGTGCAA AACAGGAGGC ACATTTTCCC  
3721 CACCTGTGTA GGTTCACAAA TATCTAGTGT TTTTATTTT ACTTGATCA GGAACCCAGC  
3781 ACTCCACTGG ATAAGCATTA TCCTTATCCA AAACAGCCTT GTGGTCAGTG TTCATCTGCT  
3841 GACTGTCAAC TGTAGCATTT TTTGGGGTTA CAGTTTGAGC AGGATATTTG GTCCTGTAGT  
3901 TTGCTAACAC ACCCTGCAGC TCCAAAGGTT CCCACCAAC AGCAAAAAA TGAAATTTG  
3961 ACCCTTGAAT GGGTTTTCCA GCACCATTTT CATGAGTTT TTGTGTCCCT GAATGCAAGT  
4021 TTAACATAGC AGTTACCCCA ATAACCTCAG TTTTAAAGT AACAGCTTCC CACATCAAAA  
4081 TATTTCCACA GGTAAAGTCC TCATTTAAAT TAGGCAAGG AATTGCTCTA GAGCGGCCGC  
4141 CACCGCGGTG GAGCTCCAAT TCGCCCTATA GTGAGTCGTA TTACGCGCGC TCACTGGCCG  
4201 TCGTTTTACA ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG  
4261 CACATCCCCC TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCAGT CGCCCTTCCC  
4321 AACAGTTGCG CAGCCTGAAT GCGGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG  
4381 CGGGTGTGGT GGTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC  
4441 CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCCCGG CTTTCCCGT CAAGCTCTAA  
4501 ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAC  
4561 TTGATTAGGG TGATGGTTCA CGTAGTGGG CATCGCCCTG ATAGACGGTT TTTGCGCCCT  
4621 TGACGTGGA GTCCACGTTT TTAATAGTG GACTCTTGT CCAAAGTGA ACAACTCA  
4681 ACCCTATCTC GGTCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTG GCCTATTGGT  
4741 TAAAAAATGA GCTGATTTAA CAAAAATTA ACGCGAATTT TAACAAAATA TTAACGCTTA  
4801 CAATTTAGGT GGCATTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTCTA  
4861 AATACATTC AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC TTCAATAATA  
4921 TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTGTG  
4981 GGCATTTTGC CTTCTGTTT TTGCTCACC AGAAACGCTG GTGAAAGTAA AAGATGCTGA  
5041 AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAAGTGGAT CTCAACAGCG GTAAGATCCT  
5101 TGAGAGTTT CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG  
5161 TGGCGCGGTA TTATCCCGTA TTGACGCGG GCAAGAGCAA CTCGGTCGCC GCATACACTA  
5221 TTCTCAGAA GACTTGTTG AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT  
5281 GACAGTAAGA GAATTATGCA GTGCTGCCAT AACCATGAGT GATAACACTG CGGCCAACTT  
5341 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCCTG TTTTGCACA ACATGGGGGA  
5401 TCATGTAAC CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA  
5461 GCGTGACACC ACGATGCCTG TAGCAATGGC AACACGTTG CGCAAATAT TAACTGGCGA  
5521 ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC  
5581 AGGACCACTT CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA AATCTGGAGC  
5641 CGGTGAGCGT GGGTCTCGCG GTATCATTC AGCACTGGGG CCAGATGGTA AGCCCTCCCG  
5701 TATCGTAGTT ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT  
5761 CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG TTTACTCATA  
5821 TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG TGAAGATCCT  
5881 TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT GAGCGTCAGA  
5941 CCCCCTAGAA AAGATCAAAG GATCTTCTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG  
6001 CTTGCAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGATC AAGAGCTACC-

FIGURE 31C

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6061 AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA CTGTCCTTCT  
6121 AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA CATACTCGC  
6181 TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC TTACCGGGTT  
6241 GGA CTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTTCGTG  
6301 CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT  
6361 ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG TAAGCGGCAG  
6421 GGTCCGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT ATCTTTATAG  
6481 TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT CGTCAGGGGG  
6541 GCGGAGCCTA TGGAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCTTGG CCTTTTGCTG  
6601 GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC  
6661 CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA GCGAGTCAGT  
6721 GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT CTCCCCGCGC GTTGGCCGAT  
6781 TCATTAATGC AGCTGGCAGC ACAGGTTTCC CGACTGGAAA GCGGGCAGTG AGCGCAACGC  
6841 AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC  
6901 TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTAC ACAGGAAACA GCTATGACCA  
6961 TGATTACGCC AAGCGCGCAA TTAACCCTCA CTAAAGGGAA CAAAAGCTGG GTACCGGGCC  
7021 CCCCCCT

FIGURE 31D

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**Figure 32A: pDEST12.2 CMV Promoter for Eukaryotic Expression, SV40 Promoter/ori for G418 Resistance**

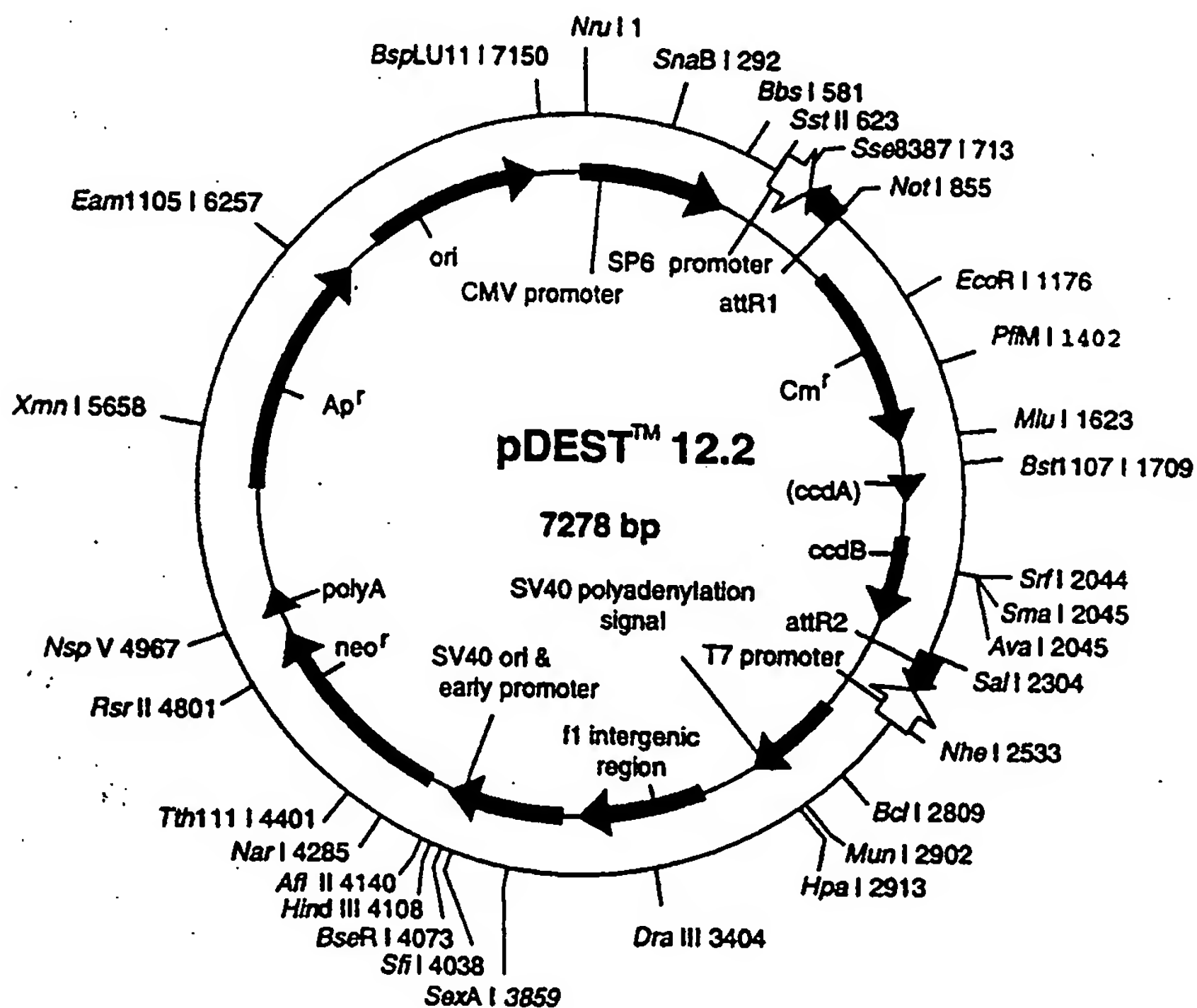
307 *mRNA from CMV promoter*  
acc gtc aga tgc cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa  
tgg cag tct agc gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt

358 gac acc ggg acc gat cca gcc tcc gga ctc tag cct agg ccg cgg agc gga  
ctg tgg ccc tgg cta ggt cgg agg cct gag atc gga tcc ggc gcc tgc cct

409 taa caa ttt cac aca gga aac agc tat gac cat tag gcc ttt gca aaa agc  
att gtt aaa gtg tgt cct ttg tgc ata ctg gta atc cgg aaa cgt ttt tgc

460 tat tta ggt gac act ata gaa ggt acg cct gca ggt *Age* *EcoRI*  
ata aat cca ctg tga tat ctt cca tgc gga cgt cca tgg cca ggc ctt aag  
*Int* *attR1*

511 cca tca *aca agt tgg taa ada ada gct gaa cga gaa acg taa aat gat ata*  
ggt agt *tgt tca aac atg ttt ttt cga cct gct ctt tgc att gta cta tat*



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## pDEST12.2 7278 bp (rotated to position 3900)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
86..136	ori
220..742	CMV promoter
1059..935	attR1
1168..1827	CmR
1947..2031	inactivated ccdA
2169..2474	ccdB
2515..2639	attR2
2824..3186	small t & polyA
3310..3378	lac
4363..5157	neo
5680..6540	ampR

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1  GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT
61 TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT
121 ATTACCGCCT TTGAGTGAGC TGATACCGCT CGCCGCAGCC GAACGACCGA GCGCAGCGAG
181 TCAGTGAGCG AGGAAGCGGA AGAGCTCGCG AATGCATGTC GTTACATAAC TTACGGTAAA
241 TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT
301 TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT ATTTACGGTA
361 AACTGCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT
421 CAATGACGGT AAATGGCCCG CCTGGCATTG TGCCAGTAC ATGACCTTAT GGGACTTTCC
481 TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA
541 GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT
601 TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA AATGTCGTAA
661 CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG
721 CAGAGCTCGT TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT
781 CCATAGAAGA CACCGGGACC GATCCAGCCT CCGGACTCTA GCCTAGGCCG CGGGACGGAT
841 AACAAATTTCA CACAGGAAAC AGCTATGACC ATTAGGCCTT TGCAAAAAGC TATTTAGGTG
901 AACTATAGA AGGTACGCCT GCAGGTACCG GATCACAAGT TTGTACAAA AAGCTGAACG
961 AGAAACGTAA AATGATATAA ATATCAATAT ATTAAATTAG ATTTTGCATA AAAAACAGAC
1021 TACATAATAC TGTAACACAC AACATATCCA GTCATATGCG CGGCCGCATT AGGCACCCCA
1081 GGCTTTACAC TTTATGCTTC CGGCTCGTAT AATGTGTGGA TTTTGAGTTA GGATCCGTCG
1141 AGATTTTCAG GAGCTAAGGA AGCTAAAATG GAGAAAAAAA TCACTGGATA TACCACCGTT
1201 GATATATCCC AATGGCATCG TAAAGAACAT TTTGAGGCAT TTCAGTCAGT TGCTCAATGT
1261 ACCTATAACC AGACCGTTCA GCTGGATATT ACGGCCTTTT TAAAGACCGT AAAGAAAAAT
1321 AAGCACAAGT TTTATCCGGC CTTTATTAC ATTCCTGCCC GCCTGATGAA TGCTCATCCG
1381 GAATTCGTA TGGCAATGAA AGACGGTGAG CTGGTGATAT GGGATAGTGT TCACCCTTGT
1441 TACACCGTTT TCCATGAGCA AACTGAAACG TTTTCATCGC TCTGGAGTGA ATACCACGAC
1501 GATTTCCGGC AGTTTCTACA CATATATTTC CAAGATGTGG CGTGTTACGG TGAAAACCTG
1561 GCCTATTTCC CTAAAGGGTT TATTGAGAAT ATGTTTTTCG TCTCAGCAA TCCCTGGGTG
1621 AGTTTCACCA GTTTTGATTT AAACGTGGCC AATATGGACA ACTTCTTCGC CCCCGTTTTT
1681 ACCATGGGCA AATATTATAC GCAAGGCGAC AAGGTGCTGA TGCCGCTGGC GATTCAGGTT
1741 CATCATGCCG TCTGTGATGG CTTCCATGTC GGCAGAATGC TTAATGAATT ACAACAGTAC
1801 TGCGATGAGT GGCAGGGCGG GGCCTAAACG CGTGGATCCG GCTTACTAAA AGCCAGATAA
1861 CAGTATGCGT ATTTGCGCGC TGATTTTTTC GGTATAAGAA TATATACTGA TATGTATACC
1921 CGAAGTATGT CAAAAGAGG TGTGCTATGA AGCAGCGTAT TACAGTGACA GTTGACAGCG
1981 ACAGCTATCA GTTGCTCAAG GCATATATGA TGTCAATATC TCCGGTCTGG TAAGCACAAC
2041 CATGCAGAAT GAAGCCCGTC GTCTGCGTGC CGAACGCTGG AAAGCGGAAA ATCAGGAAGG
2101 GATGGCTGAG GTCGCCCCGT TTATTGAAAT GAACGGCTCT TTTGCTGACG AGAACAGGGA
2161 CTGGTGAAAT GCAGTTTAAG GTTTACACCT ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG
2221 TGGATGTACA GAGTGATATT ATTGACACGC CCGGGCGACG GATGGTGATC CCCCTGGCCA
2281 GTGCACGTCT GCTGTCAGAT AAAGTCTCCC GTGAACTTTA CCCGGTGGTG CATATCGGGG
2341 ATGAAAGCTG GCGCATGATG ACCACCGATA TGGCCAGTGT GCCGGTCTCC GTTATCGGGG
2401 AAGAAGTGGC TGATCTCAGC CACCGCGAAA ATGACATCAA AAACGCCATT AACCTGATGT-

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FIGURE 32B



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2461 TCTGGGGAAT ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT  
2521 GACTGGATAT GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT  
2581 AATATATTGA TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTG  
2641 ATCGCGTGCA TCGGACGTCA TAGCTCTCTC CCTATAGTGA GTCGTATTAT AAGCTAGGCA  
2701 CTGGCCGTCG TTTTACAACG TCGTGAAGTGG GAAAACTGCT AGCTTGGGAT CTTTGTGAAG  
2761 GAACCTTACT TCTGTGGTGT GACATAATTG GACAACTAC CTACAGAGAT TTAAAGCTCT  
2821 AAGGTAAATA TAAAATTTTT AAGTGTATAA TGTGTTAAAC TAGCTGCATA TGCTTGCTGC  
2881 TTGAGAGTTT TGCTTACTGA GTATGATTTA TGAAAATATT ATACACAGGA GCTAGTGATT  
2941 CTAATTGTTT GTGTATTTTA GATTCACAGT CCCAAGGCTC ATTTTCAGGCC CCTCAGTCCT  
3001 CACAGTCTGT TCATGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA  
3061 AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTTGTTT  
3121 AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTTACA  
3181 AATAAAGCAT TTTTTCCTACT GCATTCCTAGT TGTGGTTTTGT CCAAACCTCAT CAATGTATCT  
3241 TATCATGTCT GGATCGATCC TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGTTT  
3301 GCGTATTGGC TGGCGTAATA GCGAAGAGGC CCGCACCAGT CGCCCTTCCC AACAGTTGCG  
3361 CAGCCTGAAT GGCGAATGGG ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT  
3421 GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT  
3481 CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT  
3541 CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTAGGG  
3601 TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCCGCCCT TGACGTTGGA  
3661 GTCCACGTTT TTTAATAGTG GACTCTTGTT CCAAACCTGGA ACAACACTCA ACCCTATCTC  
3721 GGTCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTCT GCCTATTGGT TAAAAAATGA  
3781 GCTGATTTAA CAAATATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTCCC  
3841 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA CGCGGATCTG  
3901 CGCAGCACCA TGGCCTGAAA TAACCTCTGA AAGAGGAACT TGGTTAGGTA CCTTCTGAGG  
3961 CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC  
4021 AGCAGGCAGA AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC  
4081 CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAT  
4141 AGTCCCGCCC CTAACCTCCG CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC  
4201 GCCCCATGGC TGACTAATTT TTTTATTTA TGCAGAGGCC GAGGCCGCCT CGGCTCTGTA  
4261 GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTTGA  
4321 TTCTTCTGAC ACAACAGTCT CGAACTTAAG GCTAGAGCCA CCATGATTGA ACAAGATGGA  
4381 TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA CTGGGCACAA  
4441 CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT CAGCGCAGGG GCGCCCGGTT  
4501 CTTTTTGTC AAGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGACGA GGCAGCGCGG  
4561 CTATCGTGGC TGGCCACGAC GGGCGTTCCT TGCGCAGCTG TGCTCGACGT TGTCACTGAA  
4621 GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC AGGATCTCCT GTCATCTCAC  
4681 CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA TGCGGCGGCT GCATACGCTT  
4741 GATCCGGCTA CCTGCCCATT CGACCACCAA GCGAAACATC GCATCGAGCG AGCACGTACT  
4801 CCGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG AAGAGCATCA GGGGCTCGCG  
4861 CCAGCCGAAC TGTTCCGCCAG GCTCAAGGCG CGCATGCCCC ACGGCGAGGA TCTCGTCGTG  
4921 ACCCATGGCG ATGCCTGCTT GCCGAATATC ATGGTGGAAT ATGGCCGCTT TTCTGGATTCT  
4981 ATCGACTGTG GCGGGCTGGG TGTGGCGGAC CGCTATCAGG ACATAGCGTT GGCTACCCGT  
5041 GATATTGCTG AAGAGCTTGG CCGCGAATGG GCTGACCGCT TCCTCGTGCT TTACGGTATC  
5101 GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTTC TTGACGAGTT CTTCTGAGCG  
5161 GGAATCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA CCTGCCATCA CGATGGCCGC  
5221 AATAAAATAT CTTTATTTTC ATTACATCTG TGTGTTGGTT TTTTGTGTGA ATCGATAGCG  
5281 ATAAGGATCC GCGTATGGTG CACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC  
5341 CAGCCCCGAC ACCCGCCAAC ACCCGCTGAC GCGCCCTGAC GGGCTTGTCT GCTCCCGGCA  
5401 TCCGCTTACA GACAAGCTGT GACCGTCTCC GGGAGCTGCA TGTGTCAGAG GTTTTCACCG  
5461 TCATCACCAG AACGCGCGAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT  
5521 GTCATGATAA TAATGGTTTT TTAGACGTCA GGTGGCACTT TTCGGGGAAA TGTGCGCGGA  
5581 ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA  
5641 CCCTGATAAA TGCTTCAATA ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT  
5701 GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCCTTCTG TTTTGTGCTA CCCAGAAACG  
5761 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG  
5821 GATCTCAACA GCGGTAAAGT CCTTGAGAGT TTTCCGCCCC AAGAACGTTT TCCAATGATG  
5881 AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTATTGACGC CGGGCAAGAG-

FIGURE 32C

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5941 CAACTCGGTC GCCGCATACA CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA  
6001 GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG  
6061 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC  
6121 GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG ATCGTTGGGA ACCGGAGCTG  
6181 AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CTGTAGCAAT GGCAACAACG  
6241 TTGCGCAAAC TATTAAGTGG CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC  
6301 TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG  
6361 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG  
6421 GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA CGACGGGGAG TCAGGCAACT  
6481 ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA  
6541 CTGTCAGACC AAGTTTACTC ATATATACTT TAGATTGATT TAAAACTTCA TTTTAAATTT  
6601 AAAAGGATCT AGGTGAAGAT CCTTTTGTAT AATCTCATGA CCAAAATCCC TTAACGTGAG  
6661 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT  
6721 TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT  
6781 TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG  
6841 CAGATACCAA ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT  
6901 GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC  
6961 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG  
7021 TCGGGCTGAA CGGGGGGFTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA  
7081 CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG  
7141 GACAGGTATC CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG  
7201 GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCTGA  
7261 TTTTTGTGAT GCTCGTCA

FIGURE 32D



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Figure 33A:

pDEST13

Native protein in E. coli:  $\lambda$ PL  
promoter

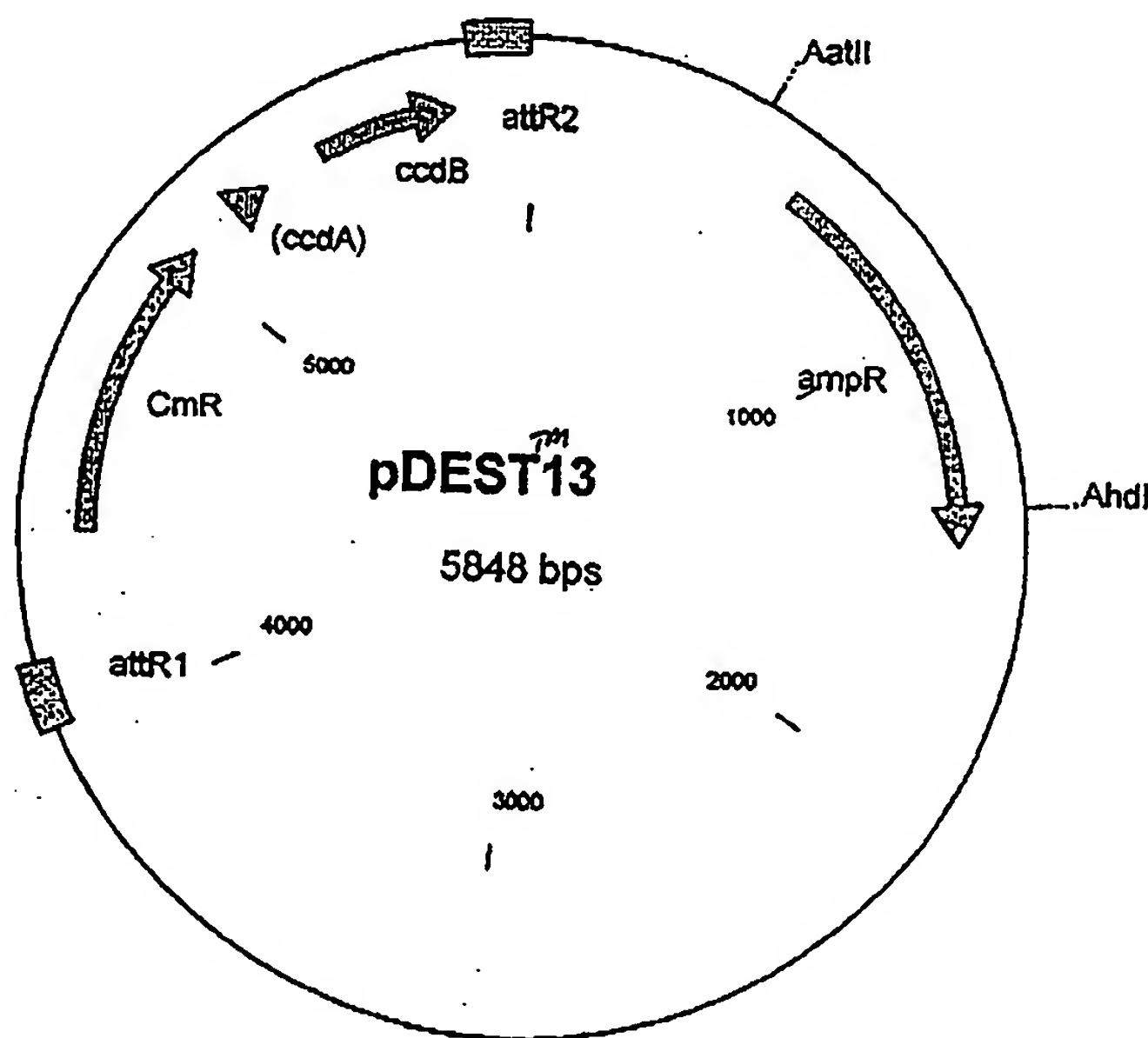
3721 tgggcaaacc aagacagcta aagatctctc acctaccaaa caatgcccc ctgcaaaaa  
 acccgtttgg ttctgtcgat ttctagagag tggatgggtt gttacggggg gacgtttttt

3781 taaattcata taaaaaacat acagataacc atctgcggtg ataaattatc tctggcggtg  
 atttaagtat attttttgta tgtctattgg tagacgccac tatttaatag agaccgccac

3841 ttgacataaa taccactggc ggtgatactg agcacatcag caggacgcac tgaccaccat  
aactgtattt atggtgaccg ccactatgac tctgttagtc gtctgcgtg actggtggta

3901 gaaggtgacg ctcttaaaaa ttaagecctg aagaaggcca gcattcaaag cagaaggctt  
 cttccactgc gagaattttt aattcgggac ttcttcccggt cgtaagtctt gtcttccgaa

3961 tggggtgtgt gatacgaaac gaagcattgg gatcatcaca agtttgtaca aaaaagctga  
 accccacaca ctatgctttg ctcgtaacc ctagtagtgt tcaaacatgt ttttccgact



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## pDEST13 5848 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
599..1458		ampR
4123..3998		attR1
4372..5031		CmR
5151..5235		inactivated ccdA
5373..5678		ccdB
5719..5843		attR2

1	TTC	ACT	TGG	CC	GTC	GTT	TTT	TAC	AAC	GTC	CGT	GTA	CTG	GGA	AAA	AC	CCT	GGC	GTT	AA
61	TCG	CCT	TG	CA	GC	AC	AT	CCCC	CTT	TC	GCC	CAG	CTG	GCG	TA	AT	AG	CGA	AAG	AGG
121	TCG	CC	CTT	CC	CA	AC	AG	TTG	GC	AG	CCT	GAA	TGG	CGA	AT	GG	CG	CT	GAT	GCG
181	CCT	TAC	GC	CAT	CT	GT	GCG	GTA	TTT	CAC	AC	CG	CAT	AT	TGG	TGC	ACT	CT	CAG	TAA
241	TG	AT	GCC	GCA	TAG	TTA	AG	CC	AG	CCCC	GACA	CCC	GCA	ACA	CCC	GCT	GAC	G	CG	CC
301	GG	CTT	GT	CTG	CT	CCC	G	GCAT	CCG	CTT	TAC	AG	ACA	AG	CTG	TG	ACC	GT	CT	CCG
361	GT	GT	CAG	AGG	TTTT	CAC	CGT	CAT	CAC	CGAA	ACG	CG	CGA	GAG	CGAA	AGG	GCC	TC	GT	GAT
421	CCT	AT	TTTT	TTA	TAG	GTT	TA	TG	TCAT	GATA	TAAT	AAT	G	GGT	TTT	CT	TAG	AC	GT	CAG
481	TCG	GGG	GAA	AT	GT	GCG	CG	GAA	CCC	CT	AT	TTG	TTT	AT	TTTT	CT	TAA	ATA	CATT	CAA
541	TCC	GCT	CAT	G	AG	ACA	ATA	AC	CCT	GATA	TAAT	G	CTT	CA	ATAA	TAT	TG	AAAA	GGA	AG
601	GAG	TAT	TCAA	CAT	TTT	CC	G	TG	TCG	CC	CTT	AT	TCC	CT	TTTT	G	CGG	CA	TTTT	GC
661	TTTT	GCT	CAC	CC	AG	AA	AC	GC	TGG	TG	AA	AGT	AAA	AG	AT	GCT	GA	AG	AT	CAG
721	AG	TGG	GTT	AC	AT	CG	AA	CTGG	AT	CT	CA	AC	AG	CGG	TA	AG	AT	C	AGT	TGG
781	AGA	AC	GTTTT	CCA	AT	GAT	G	GA	GCA	CTT	TTT	AA	AGT	TCT	GCTA	TGT	G	CG	CGG	TAT
841	TAT	TG	AC	GCC	GGG	CA	AG	AGC	AAC	T	CGG	T	CG	CCG	CAT	AC	AT	TCT	CAG	ATG
901	TG	AG	TACT	CA	CC	AG	T	CAC	AG	AAA	AG	CAT	CT	TAC	G	GAT	TGG	AT	G	AGTAA
961	CAG	TG	CT	GCC	ATA	ACC	AT	G	GT	GATA	AA	CAC	TG	CGG	CCA	AC	TT	ACT	TCT	G
1021	AGG	ACC	GAA	AG	GAG	CTA	ACC	G	CTT	TTT	TG	CA	CA	AC	AT	G	G	AT	GTAA	CTC
1081	TCG	TT	G	GAA	CCG	GAG	CT	G	AT	G	AG	CCAT	ACC	AA	AC	GAC	GAG	CG	TG	ACA
1141	TG	TAG	CA	ATG	GCA	ACA	AC	G	TG	CG	CA	AA	CT	AT	TAA	CT	G	GA	ACT	TTA
1201	CCG	GCA	ACAA	TTA	AT	AG	ACT	GG	AT	G	GAG	GC	GG	ATA	AA	AGT	GC	AG	GACC	AC
1261	GG	CC	TTCC	G	CT	G	GCT	G	TT	AT	TG	CTG	TA	AA	AT	CT	GGA	GCC	GT	G
1321	CGG	TAT	CATT	GC	AG	CA	CTGG	GG	CC	AG	ATGG	TA	AG	CC	CTCC	CG	TAT	CG	TAG	TTAT
1381	GAC	G	G	GAGT	CAG	GCA	ACTA	TGG	AT	GAA	ACG	AA	AT	AG	ACAG	AT	CG	CT	GAG	TAG
1441	ACT	GAT	TAA	G	CAT	TG	GTA	AC	TG	T	CAG	ACCA	AG	TTT	ACT	CA	TAT	ATA	CTTT	AG
1501	AAA	ACT	TCAT	TTTT	AA	TTT	TA	TTA	AA	AG	GAT	CTA	GG	TGA	AG	ATC	CTT	TTT	GATA	ATCT
1561	CAA	AA	TCCCT	TA	AC	GT	GAGT	TTT	CG	TT	TCCA	CT	GAG	CGT	CA	GAC	CC	CGT	AG	AAA
1621	AGG	AT	CTTCT	TG	AG	AT	CCTT	TTTT	TCT	GCG	CG	TA	AT	CT	G	TG	CTT	G	CAAA	CAAAA
1681	ACC	GCT	ACCA	GCG	G	TG	GTTT	G	TTT	TG	CCGGA	TCA	AG	AG	CTA	CCA	ACT	CTTT	TT	CCGA
1741	AA	CTG	GCTTC	AG	CAG	AG	CGC	AG	ATA	ACCA	AA	TAC	TG	TTCT	CTT	CTAG	T	G	TAGC	CGTAG
1801	CC	ACC	ACTTC	AAG	AA	CTCTG	TAG	CAC	CGCC	TAC	ATA	CTC	GCT	CT	GCTAA	TC	CT	GTT	TACC	
1861	AG	TGG	CTGCT	GCC	AG	TGG	CG	ATA	AGT	CGTG	TCTT	ACC	GGG	TTG	GACT	CAA	GAC	GAT	AGTT	
1921	ACC	G	GATAAG	GCG	CAG	CGGT	CGG	GCT	GAA	AC	GGG	G	G	G	G	G	TG	CAC	ACAG	CCAG
1981	GCG	AAC	GACC	TAC	ACC	GAA	AC	TG	AG	ATACCT	AC	AG	CGT	GAG	CATT	GAG	AAA	GCG	CCAC	GCT
2041	TCC	CGA	AGGG	AG	AA	AGG	CGG	AC	AGG	TATCC	GG	TA	AG	CGG	AG	GGT	CG	GAA	CAG	GAG
2101	CAC	GAG	GGGAG	CTT	CC	AGGGG	GAA	ACG	CCTG	GT	ATCT	TTAT	AG	TCT	GTCG	GG	TTT	CG	CCA	
2161	CCT	CT	GACTT	GAG	CGT	CGAT	TTTT	TG	TATG	CTCGT	CAGG	GGG	CGG	AG	CC	TAT	G	AAAA	AAAA	
2221	CGC	CAG	CAAC	GCG	GCT	TTTT	TAC	G	GTT	CTCT	GGC	CTT	TG	TGG	CT	TTTT	CT	CAC	ATGTT	
2281	CTT	TCT	GCG	TT	AT	CCCCTG	ATT	CTG	TGGA	TA	ACCG	TATT	ACC	G	CTTTG	AGT	GAG	CTGA		
2341	TAC	CGCT	CGC	CG	CAG	CCGAA	CG	ACC	GAGCG	CAG	CGAGT	CA	GT	GAG	CGAGG	AAG	CGGA	AGA		
2401	GCG	CCA	ATA	CG	CAA	ACCGC	CT	CT	CCCCG	GCG	TTG	GCCG	ATT	CATT	TAAT	GC	AG	CTGG	CA	
2461	CG	AC	AGTTT	CCC	GACT	GGA	AAG	CGG	G	CAG	TGAG	CGCA	AC	GCA	ATT	TAATG	TG	AGTT	AGCT	
2521	CAC	T	CATTAG	GC	AC	CC	CAGG	CTT	TAC	ACTT	TAT	GCTT	CCG	GCT	CGT	ATGT	TGT	TG	GGAAT	
2581	TGT	GAG	CGGA	TA	ACA	ATTTT	AC	AC	AGGAA	ACAG	CTAT	GAC	CAT	GATT	ACG	CCA	AGCT	TGG		
2641	CTG	CAG	G	TGA	TG	ATTAT	CAG	CC	AG	CAGAGA	TTA	AGG	AAA	CAG	AC	AGGTT	TAT	TG	AGCGC	
2701	TT	AT	CTTTCC	CTT	TAT	TTTTT	GCT	GCG	G	TAA	GTC	G	CATA	AA	AACC	ATTCTT	CATA	ATT	CAA-	

FIGURE 33B

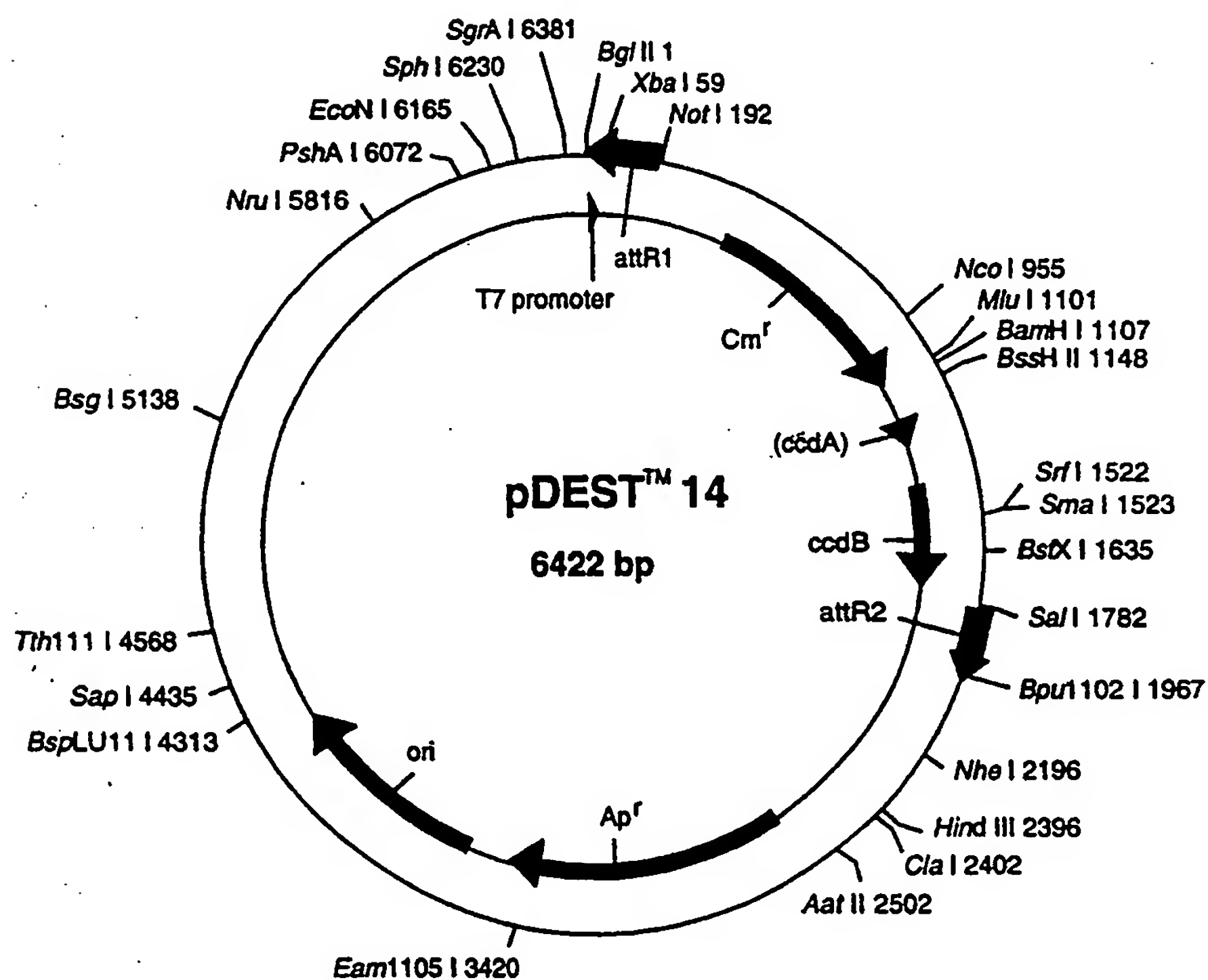
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2761 TCCATTTACT ATGTTATGTT CTGAGGGGAG TGAAAATTCC CCTAATTCGA TGAAGATTCT  
2821 TGCTCAATTG TTATCAGCTA TGCGCCGACC AGAACACCTT GCCGATCAGC CAAACGTCTC  
2881 TTCAGGCCAC TGACTAGCGA TAACTTTCCC CACAACGGAA CAACTCTCAT TGCATGGGAT  
2941 CATTGGGTAC TGTGGGTTTA GTGGTTGTAA AAACACCTGA CCGCTATCCC TGATCAGTTT  
3001 CTTGAAGGTA AACTCATCAC CCCCAAGTCT GGCTATGCAG AAATCACCTG GCTCAACAGC  
3061 CTGCTCAGGG TCAACGAGAA TTAACATTCC GTCAGGAAAG CTTGGCTTGG AGCCTGTTGG  
3121 TGCGGTCATG GAATTACCTT CAACCTCAAG CCAGAATGCA GAATCACTGG CTTTTTTGGT  
3181 TGTGCTTACC CATCTCTCCG CATCACCTTT GGTAAAGGTT CTAAGCTTAG GTGAGAACAT  
3241 CCCTGCCTGA ACATGAGAAA AAACAGGGTA CTCATACTCA CTTCTAAGTG ACGGCTGCAT  
3301 ACTAACCGCT TCATACATCT CGTAGATTTC TCTGGCGATT GAAGGGCTAA ATTCTTCAAC  
3361 GCTAACTTTG AGAATTTTTG CAAGCAATGC GGC GTTATAA GCATTTAATG CATTGATGCC  
3421 ATTAAATAAA GCACCAACGC CTGACTGCCC CATCCCCATC TTGTCTGCGA CAGATTCCTG  
3481 GGATAAGCCA AGTTCATTTT TCTTTTTTTC ATAAATTGCT TTAAGGCGAC GTGCGTCCTC  
3541 AAGCTGCTCT TGTGTTAATG GTTCTTTTTT TGTGCTCATA CGTTAAATCT ATCACCGCAA  
3601 GGGATAAATA TCTAACACCG TGCGTGTTGA CTATTTTACC TCTGGCGGTG ATAATGGTTG  
3661 CATGTACTAA GGAGGTTGTA TGGAAACAACG CATAACCCTG AAAGATTATG CAATGCGCTT  
3721 TGGGCAAACC AAGACAGCTA AAGATCTCTC ACCTACCAA CAATGCCCCC CTGCAAAAAA  
3781 TAAATTCATA TAAAAACAT ACAGATAACC ATCTGCGGTG ATAAATTATC TCTGGCGGTG  
3841 TTGACATAAA TACCACTGGC GGTGATACTG AGCACATCAG CAGGACGCAC TGACCACCAT  
3901 GAAGGTGACG CTCTTAAAAA TTAAGCCCTG AAGAAGGGCA GCATTCAAAG CAGAAGGCTT  
3961 TGGGGTGTGT GATACGAAAC GAAGCATTGG GATCATCACA AGTTTGTACA AAAAAGCTGA  
4021 ACGAGAAACG TAAAATGATA TAAATATCAA TATATTAAAT TAGATTTTGC ATAAAAACA  
4081 GACTACATAA TACTGTAAAA CACAACATAT CCAGTCACTA TGGCGGCCGC TAAGTTGGCA  
4141 GCATCACCCG ACGCACTTTG CGCCGAATAA ATACCTGTGA CGGAAGATCA CTTGCGAGAA  
4201 TAAATAAATC CTGGTGTCCC TGTTGATACC GGAAGCCCT GGGCCAACTT TTGGCGAAAA  
4261 TGAGACGTTG ATCGGCACGT AAGAGGTTCC AACTTTCACC ATAATGAAAT AAGATCACTA  
4321 CCGGGCGTAT TTTTGTAGTT ATCGAGATTT TCAGGAGCTA AGGAAGCTAA AATGGAGAAA  
4381 AAAATCACTG GATATACCAC CGTTGATATA TCCCAATGGC ATCGTAAAGA ACATTTTGAG  
4441 GCATTTTCAGT CAGTTGCTCA ATGTACCTAT AACCAGACCG TTCAGCTGGA TATTACGGCC  
4501 TTTTAAAGA CCGTAAAGAA AAATAAGCAC AAGTTTTATC CGGCCTTTAT TCACATTCTT  
4561 GCGCGCCTGA TGAATGCTCA TCCGGAATTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG  
4621 ATATGGGATA GTGTTACCCC TTGTTACACC GTTTTCCATG AGCAAACCTGA AACGTTTTCA  
4681 TCGCTCTGGA GTGAATACCA CGACGATTTT CCGCAGTTTC TACACATATA TTCGCAAGAT  
4741 GTGGCGTGTT ACGGTGAAAA CCTGGCCTAT TTCCCTAAAG GGTTTATTGA GAATATGTTT  
4801 TTCGTCTCAG CCAATCCCTG GGTGAGTTTC ACCAGTTTTG ATTTAAACGT GGCCAATATG  
4861 GACAACTTCT TCGCCCCCGT TTTCACCATG GGCAAATATT ATACGCAAGG CGACAAGGTG  
4921 CTGATGCCGC TGGCGATTCA GGTTCATCAT GCCGTCTGTG ATGGCTTCCA TGTCGGCAGA  
4981 ATGCTTAATG AATTACAACA GTACTGCGAT GAGTGGCAGG GCGGGGCGTA AACGCGTGGA  
5041 TCCGGCTTAC TAAAAGCCAG ATAACAGTAT GCGTATTTGC GCGCTGATTT TTGCGGTATA  
5101 AGAATATATA CTGATATGTA TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC  
5161 GTATTACAGT GACAGTTGAC AGCGACAGCT ATCAGTTGCT CAAGGCATAT ATGATGTCAA  
5221 TATCTCCGGT CTGGTAAGCA CAACCATGCA GAATGAAGCC CGTCGTCTGC GTGCCGAACG  
5281 CTGGAAAGCG GAAAATCAGG AAGGGATGGC TGAGGTGCGC CGGTTTATTG AAATGAACGG  
5341 CTCTTTTGCT GACGAGAACA GGGACTGGTG AAATGCAGTT TAAGGTTTAC ACCTATAAAA  
5401 GAGAGAGCCG TTATCGTCTG TTTGTGGATG TACAGAGTGA TATTATTGAC ACGCCGGGC  
5461 GACGGATGGT GATCCCCCTG GCCAGTGCAC GTCTGCTGTC AGATAAAGTC TCCCGTGAAC  
5521 TTTACCCGGT GGTGCATATC GGGGATGAAA GCTGGCGCAT GATGACCACC GATATGGCCA  
5581 GTGTGCCGGT CTCCGTTATC GGGGAAGAAG TGGCTGATCT CAGCCACCGC GAAAATGACA  
5641 TCAAAAACGC CATTAACTG ATGTTCTGGG GAATATAAAT GTCAGGCTCC GTTATACACA  
5701 GCCAGTCTGC AGGTGACCA TAGTGACTGG ATATGTTGTG TTTTACAGTA TTATGTAGTC  
5761 TGTTTTTTAT GCAAAATCTA ATTTAATATA TTGATATTTA TATCATTTTA CGTTTCTCGT  
5821 TCAGCTTTCT TGTACAAAGT GGTGATAA

FIGURE 33C

Figure 3<sup>4A</sup>: pDEST14 Native Protein Expression in *E. coli*, T7 Promoter

3961 tgccggccac gatgcgtccg gcgtagagga tcgagatctc gatcccgcca aattaatacg  
 acggccgggtg ctacgcaggc cgcattctct agctctagag ctagggcgct ttaattatgc  
 4021 // actcactata gggagaccac aacggtttcc ctctagatca caagtttcta caaaaaagct  
 // tgagtgatat cctctcgggtg ttgccaaagg gagatctagt gttcaaacat gtttttcga //



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## pDEST14 6422 bp (rotated to position 4000)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
185..61	attR1
435..1094	CmR
1214..1298	inactivated ccdA
1436..1741	ccdB
1782..1906	attR2
2632..3489	ampR

```
1 CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGATC
61 ACAAGTTTGT AAAAAAAGC TGAACGAGAA ACGTAAAATG ATATAAATAT CAATATATTA
121 AATTAGATTT TGCATAAAAA ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA
181 CTATGGCGGC CGCTAAGTTG GCAGCATCAC CCGACGCACT TTGCGCCGAA TAAATACCTG
241 TGACGGAAGA TCACTTCGCA GAATAAATAA ATCCTGGTGT CCCTGTTGAT ACCGGGAAGC
301 CCTGGGCCAA CTTTGGCGCA AAATGAGACG TTGATCGGCA CGTAAGAGGT TCCAACCTTC
361 ACCATAATGA AATAAGATCA CTACCGGGCG TATTTTTTGA GTTATCGAGA TTTTCAGGAG
421 CTAAGGAAGC TAAATGGAG AAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT
481 GGCATCGTAA AGAACATTTT GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA
541 CCGTTCAGCT GGATATTACG GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTTT
601 ATCCGGCCTT TATTCACATT CTGCCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG
661 CAATGAAAGA CGGTGAGCTG GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTTCC
721 ATGAGCAAAC TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT
781 TTCTACACAT ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA
841 AAGGGTTTAT TGAGAATATG TTTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAGTT
901 TTGATTTAAA CGTGGCCAAT ATGGACAAC TCTTCGCCCC CGTTTTTACC ATGGGCAAAT
961 ATTATACGCA AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTCA CATGCCGTCT
1021 GTGATGGCTT CCATGTCGGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC
1081 AGGGCGGGGC GTAAACGCGT GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT
1141 TGCGCGCTGA TTTTGGCGGT ATAAGAATAT AACTGATAT GTATACCCGA AGTATGTCAA
1201 AAAGAGGTGT GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT
1261 GCTCAAGGCA TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA
1321 GCGCGTCGTC TGCGTGCCGA ACCTGGAATA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC
1381 GCGCGGTTTA TTGAAATGAA CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA
1441 GTTTAAGGTT TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG
1501 TGATATTATT GACACGCCCC GCGACGGAT GGTGATCCCC CTGGCCAGTG CACGTCTGCT
1561 GTCAGATAAA GTCTCCCGTG AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG
1621 CATGATGACC ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA
1681 TCTCAGCCAC CGCGAAAATG ACATCAAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA
1741 AATGTCAGGC TCCCTTATAC ACAGCCAGTC TGCAGGTCGA CCATAGTGAC TGGATATGTT
1801 GTGTTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT ATATTGATAT
1861 TTATATCATT TTACGTTTCT CGTTCAGCTT TCTTGACAA AGTGGTGATG ATCCGGCTGC
1921 TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
1981 ACCCCTTGGG GCCTCTAAAC GGGTCTTGAG GGGTTTTTTG CTGAAAGGAG GAACTATATC
2041 CGGATATCCA CAGGACGGGT GTGGTCGCCA TGATCGCGTA GTCGATAGTG GCTCCAAGTA
2101 GCGAAGCGAG CAGGACTGGG CCGCGGCCAA AGCGGTCCGA CAGTGCTCCG AGAACGGGTG
2161 CGCATAGAAA TTGCATCAAC GCATATAGCG CTAGCAGCAC GCCATAGTGA CTGGCGATGC
2221 TGTCGGAATG GACGATATCC CGCAAGAGGC CCGGCAGTAC CGGCATAACC AAGCCTATGC
2281 CTACAGCATC CAGGGTGACG GTGCCGAGGA TGACGATGAG CGCATTGTTA GATTTCATAC
2341 ACGGTGCCTG ACTGCGTTAG CAATTTAACT GTGATAAACT ACCGCATTAA AGCTTATCGA
2401 TGATAAGCTG TCAAACATGA GAATTCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT
2461 TTATAGGTTA ATGTCATGAT AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA
2521 AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
2581 ATGAGACAAT AACCTTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT
2641 CAACATTTCC GTGTCGCCCT TATTCCTTTT TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT
2701 CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT
```

Figure 34B



2761 TACATCGAAC TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT  
2821 TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGTGAC  
2881 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTGAGTAC  
2941 TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG TAAGAGAATT ATGCAGTGCT  
3001 GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG  
3061 AAGGAGCTAA CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG  
3121 GAACCGGAGC TGAATGAAGC CATAACCAAC GACGAGCGTG ACACCACGAT GCCTGCAGCA  
3181 ATGGCAACAA CGTTGCGCAA ACTATTAAC TGGCGAACTAC TTAATCTAGC TTCCCGGCAA  
3241 CAATTAATAG ACTGGATGGA GCGGATAAAA GTTGCAGGAC CACTTCTGCG CTCGGCCCTT  
3301 CCGGCTGGCT GGTATTATGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC  
3361 ATTGCAGCAC TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG  
3421 AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT  
3481 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT  
3541 CATTTTAAAT TTTAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT GACCAAAATC  
3601 CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT  
3661 TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA  
3721 CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA GGTAAGTGGC  
3781 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC  
3841 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT ACCAGTGGCT  
3901 GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT  
3961 AAGGCGCAGC GGTCCGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG  
4021 ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA  
4081 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG  
4141 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG CCACCTCTGA  
4201 CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC  
4261 AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT  
4321 GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT  
4381 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCTG  
4441 ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTTAC ACCGCATATA TGGTGCATC  
4501 TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGTA TAACTCCGC TATCGCTACG  
4561 TGACTGGGTC ATGGCTGCGC CCCGACACCC GCCAACACCC GCTGACGCGC CCTGACGGGC  
4621 TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC GTCTCCGGGA GCTGCATGTG  
4681 TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCG AGTCCGTAAG GCTCATCAGC  
4741 GTGGTCGTGA AGCGATTAC AGATGTCTGC CTGTTTATCC GCGTCCAGCT CGTTGAGTTT  
4801 CTCCAGAAGC GTTAATGTCT GGCTTCTGAT AAAGCGGGCC ATGTTAAGGG CGGTTTTTTC  
4861 CTGTTTGGTC ACTGATGCCT CCGTGTAAAG GGGATTTCTG TTCATGGGGG TAATGATACC  
4921 GATGAAACGA GAGAGGATGC TCACGATACG GGTACTGAT GATGAACATG CCCGGTTACT  
4981 GGAACGTTGT GAGGGTAAAC AACTGGCGGT ATGGATGCGG CGGGACCAGA GAAAAATCAC  
5041 TCAGGGTCAA TGCCAGCGCT TCGTTAATAC AGATGTAGGT GTTCCACAGG GTAGCCAGCA  
5101 GCATCCTGCG ATGCAGATCC GGAACATAAT GGTGCAGGGC GCTGACTTCC GCGTTTCCAG  
5161 ACTTTACGAA ACACGGAAAC CGAAGACCAT TCATGTTGTT GCTCAGGTCG CAGACGTTTT  
5221 GCAGCAGCAG TCGCTTCACG TTCGCTCGCG TATCGGTGAT TCATTCTGCT AACCAGTAAG  
5281 GCAACCCCGC CAGCCTAGCC GGGTCTCAA CGACAGGAGC ACGATCATGC GCACCCGTGG  
5341 CCAGGACCCA ACGCTGCCCC AGATGCGCCG CGTGCGGCTG CTGGAGATGG CGGACGCGAT  
5401 GGATATGTTT TGCCAAGGGT TGGTTTGCGC ATTCACAGTT CTCCGCAAGA ATTGATTGGC  
5461 TCCAATTCTT GGAGTGGTGA ATCCGTTAGC GAGGTGCCGC CGGCTTCCAT TCAGGTCGAG  
5521 GTGGCCCGGC TCCATGCACC GCGACGCAAC GCGGGGAGGC AGACAAGGTA TAGGGCGGCG  
5581 CCTACAATCC ATGCCAACCC GTTCCATGTG CTCGCCGAGG CGGCATAAAT CGCCGTGACG  
5641 ATCAGCGGTC CAGTGATCGA AGTTAGGCTG GTAAGAGCCG CGAGCGATCC TTGAAGCTGT  
5701 CCCTGATGGT CGTCATCTAC CTGCCTGGAC AGCATGGCCT GCAACGCGGG CATCCCGATG  
5761 CCGCCGGAAG CGAGAAGAAT CATAATGGGG AAGGCCATCC AGCCTCGCGT CGCGAACGCC  
5821 AGCAAGACGT AGCCAGCGC GTCGGCCGCC ATGCCGGCGA TAATGGCCTG CTTCTCGCCG  
5881 AAACGTTTGG TGGCGGGACC AGTGACGAAG GCTTGAGCGA GGGCGTGCAA GATTCCGAAT  
5941 ACCGCAAGCG ACAGGCCGAT CATCGTCGCG CTCCAGCGAA AGCGGTCCTC GCCGAAATG  
6001 ACCCAGAGCG CTGCCGGCAC CTGTCCTACG AGTTGCATGA TAAAGAAGAC AGTCATAAGT  
6061 GCGGCGACGA TAGTCATGCC CCGCGCCAC CGGAAGGAGC TGACTGGGTT GAAGGCTCTC  
6121 AAGGGCATCG GTCGATCGAC GCTCTCCCTT ATGCGACTCC TGCATTAGGA AGCAGCCAG  
6181 TAGTAGGTTG AGGCCGTTGA GCACCGCCGC CGCAAGGAAT GGTGCATGCA AGGAGATGGC-



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6241 GCCCAACAGT CCCCCGGCCA CGGGGCCTGC CACCATACCC ACGCCGAAAC AAGCGCTCAT  
6301 GAGCCCGAAG TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT AGGCGCCAGC  
6361 AACCGCACCT GTGGCGCCGG TGATGCCGGC CACGATGCGT CCGGCGTAGA GGATCGAGAT  
6421 CT

FIGURE 34A

**Figure 35A: pDEST15 Glutathione-S-transferase Fusion in *E. coli*, T7 Promoter**

1 nat cga gat ctc gat ccc gcg aaa tta ata cga ctc act ata ggg aga cca  
nta gct cta gag cta ggg cgc ttt aat tat gct gag tga tat ccc tct ggt

52 caa cgg ttt ccc tct aga aat aat ttt gtt taa ctt taa gaa gga gat ata  
ggt gcc aaa ggg aga tct tta tta aaa caa att gaa att ctt cct cta tat

103 cct atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc  
gta tac agg gga tat gat cca ata acc ttt taa ttc ccg gaa cac gtt ggg

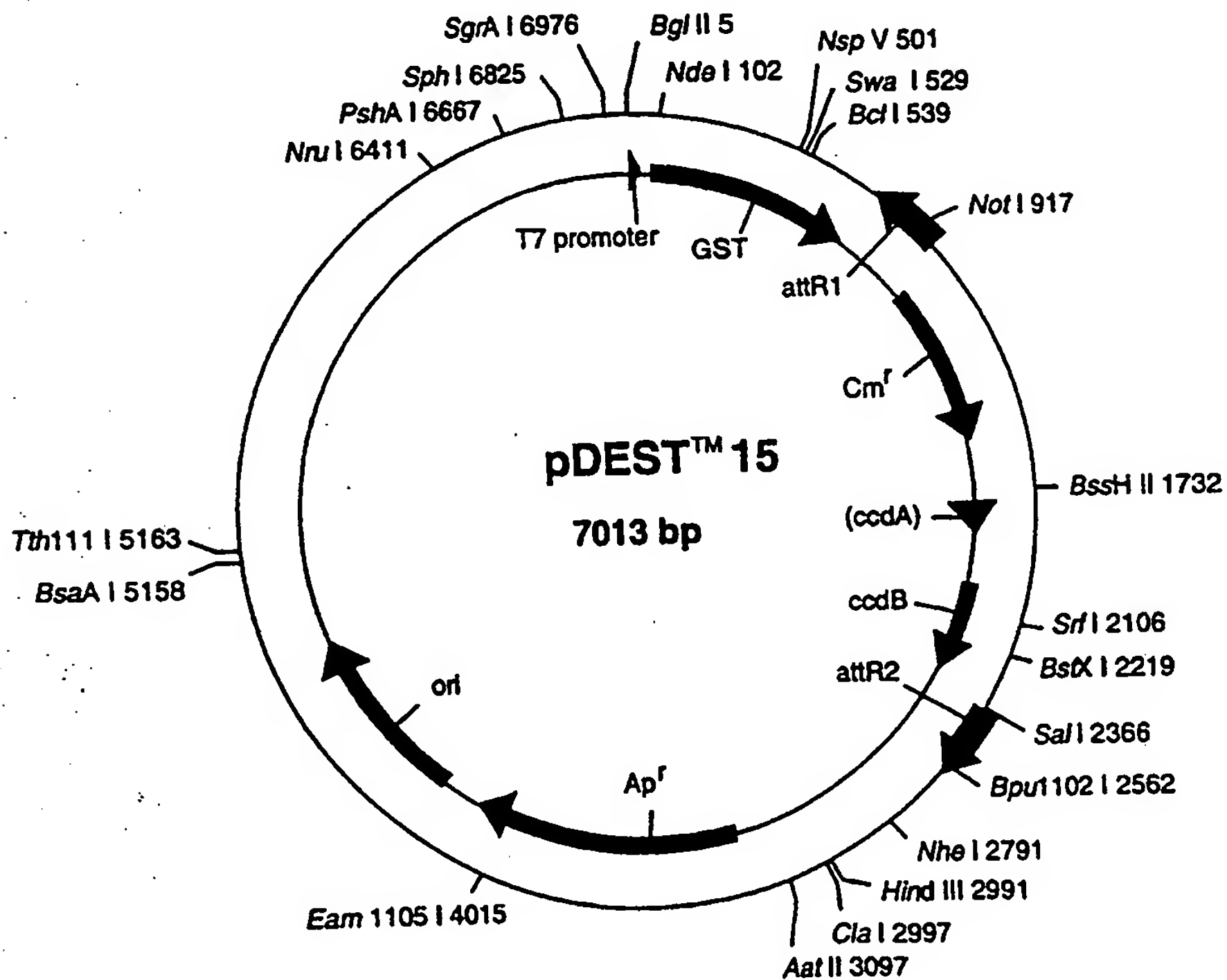
154 act cga ctt ctt ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg tat  
tga gct gaa gaa aac ctt ata gaa ctt ctt ttt ata ctt ctc gta aac ata

715 cag ggc tgg caa gcc acg ttt ggt ggt ggc gac cat cct cca aaa tcg gat  
gtc ccg acc gtt cgg tgc aaa cca cca ccg ctg gta gga ggt ttt agc cta

766 ctg gtt ccg cgt cca tgg tgc aat caa aca agt tgg tac aaa aaa gct gaa  
gac caa ggc gca ggt acc agc tta gtt tgt tca aac atg ttt ttt cga ctt

817 cga gaa acg taa aat gat ata aat atc aat ata tta aat tag att ttg cat  
gct ctt tgc att tta cta tat tta tag tta tat aat tta atc taa aac gta

*Handwritten annotations:*  
T7 Promoter (above line 1)  
mRNA (above line 1)  
XbaI (above line 52)  
NdeI (above line 103)  
Start Translation GST (below line 103)  
attR1 (below line 766)  
attR2 (below line 766)



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## pDEST15 7013 bp

Location (Base Nos.)	Gene Encoded
108..776	GST
916..792	attR1
1025..1537	CmR
1804..1888	inactivated ccdA
2026..2331	ccdB
2372..2496	attR2
3233..4093	ampR

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1 ATCGAGATCT CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC
61 CCTCTAGAAA TAATTTTGTT TAACTTTAAG AAGGAGATAT ACATATGTCC CCTATACTAG
121 GTTATTGGAA AATTAAGGGC CTTGTGCAAC CCACTCGACT TCTTTTGGAA TATCTTGAAG
181 AAAAATATGA AGAGCATTTG TATGAGCGCG ATGAAGGTGA TAAATGGCGA AACAAAAAGT
241 TTGAATTGGG TTTGGAGTTT CCCAATCTTC CTTATTATAT TGATGGTGAT GTTAAATTAA
301 CACAGTCTAT GGCCATCATA CGTTATATAG CTGACAAGCA CAACATGTTG GGTGGTTGTC
361 CAAAAGAGCG TGCAGAGATT TCAATGCTTG AAGGAGCGGT TTTGGATATT AGATACGGTG
421 TTTCGAGAAT TGCATATAGT AAAGACTTTG AAACCTCTCA AGTTGATTTT CTTAGCAAGC
481 TACCTGAAAT GCTGAAAATG TTCGAAGATC GTTTATGTCA TAAACATAT TTAAATGGTG
541 ATCATGTAAC CCATCCTGAC TTCATGTTGT ATGACGCTCT TGATGTTGTT TTATACATGG
601 ACCCAATGTG CCTGGATGCG TTCCCAAAAT TAGTTTGTTT TAAAAACGT ATTGAAGCTA
661 TCCCACAAAT TGATAAGTAC TTGAAATCCA GCAAGTATAT AGCATGGCCT TTGCAGGGCT
721 GGCAAGCCAC GTTTGGTGGT GGCGACCATC CTCCAAAATC GGATCTGGTT CCGCGTCCAT
781 GGTGCAATCA AACAAGTTTG TACAAAAAG CTGAACGAGA AACGTAAAT GATATAAATA
841 TCAATATATT AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT AAAACACAAC
901 ATATCCAGTC ACTATGGCGG CCGCATTAGG CACCCAGGC TTTACACTTT ATGCTTCCGG
961 CTCGTATAAT GTGTGGATTT TGAGTTAGGA TCCGTCGAGA TTTTCAGGAG CTAAGGAAGC
1021 TAAATGGAG AAAAAATCA CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA
1081 AGAACATTTT GAGGCATTTT AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT
1141 GGATATTACG GCCTTTTAA AGACCGTAAA GAAAAATAAG CACAAGTTT ATCCGGCCTT
1201 TATTCACATT CTGCCCCGCC TGATGAATGC TCATCCGGAA TTCCGTATGG CAATGAAAGA
1261 CGGTGAGCTG GTGATATGGG ATAGTGTTCA CCCTTGTTAC ACCGTTTTCC ATGAGCAAAC
1321 TGAAACGTTT TCATCGCTCT GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT
1381 ATATTCGCAA GATGTGGCGT GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT
1441 TGAGAAATATG TTTTCGTCT CAGCCAATCC CTGGGTGAGT TTCACCAGT TTGATTTAAA
1501 CGTGGCCAAT ATGGACAAC TCTTCGCCCC CGTTTTCCACC ATGGGCAAAT ATTATACGCA
1561 AGGCGACAAG GTGCTGATGC CGCTGGCGAT TCAGGTTTAT CATGCCGTCT GTGATGGCTT
1621 CCATGTCGGC AGAATGCTTA ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC
1681 GTAATCTAGA GGATCCGGCT TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA
1741 TTTTTCGGT ATAAGAATAT ATACTGATAT GTATACCCGA AGTATGTCAA AAAGAGGTGT
1801 GCTATGAAGC AGCGTATTAC AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA
1861 TATATGATGT CAATATCTCC GGTCTGGTAA GCACAACCAT GCAGAATGAA GCGCGTCGTC
1921 TGCGTGCCGA ACGCTGGAAA GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCGCGGTTTA
1981 TTGAAATGAA CGGCTCTTTT GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT
2041 TACACCTATA AAAGAGAGAG CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT
2101 GACACGCCCC GGCGACGGAT GGTGATCCCC CTGGCCAGTG CACGTCTGCT GTCAGATAAA
2161 GTCTCCCGTG AACTTTACCC GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC
2221 ACCGATATGG CCAGTGTGCC GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC
2281 CGCGAAAATG ACATCAAAA CGCCATTAAC CTGATGTTCT GGGGAATATA AATGTCAGGC
2341 TCCCTTATAC ACAGCCAGTC TGCAGGTCGA CCATAGTGAC TGGATATGTT GTGTTTTACA
2401 GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT ATATTGATAT TTATATCATT
2461 TTACGTTTCT CGTTCAGCTT TCTTGACAA AGTGGTTTGA TTCGACCCGG GATCCGGCTG
2521 CTAACAAAGC CCGAAAGGAA GCTGAGTTGG CTGCTGCCAC CGCTGAGCAA TAACTAGCAT
2581 AACCCCTTGG GGCCTCTAAA CGGGTCTTGA GGGGTTTTTT GCTGAAAGGA GGAAGTATAT
2641 CCGGATATCC ACAGGACGGG TGTGGTCGCC ATGATCGCGT AGTCGATAGT GGCTCCAAGT-
```

FIGURE 35B

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2701 AGCGAAGCGA GCAGGACTGG GCGGCGGCCA AAGCGGTCGG ACAGTGCTCC GAGAACGGGT  
2761 GCGCATAGAA ATTGCATCAA CGCATATAGC GCTAGCAGCA CGCCATAGTG ACTGGCGATG  
2821 CTGTCGGAAT GGACGATATC CCGCAAGAGG CCCGGCAGTA CCGGCATAAC CAAGCCTATG  
2881 CCTACAGCAT CCAGGGTGAC GGTGCCGAGG ATGACGATGA GCGCATTGTT AGATTTTCATA  
2941 CACGGTGCCT GACTGCGTTA GCAATTTAAC TGTGATAAAC TACCGCATT AAGCTTATCG  
3001 ATGATAAGCT GTCAAACATG AGAATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT  
3061 TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG  
3121 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT  
3181 CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT  
3241 TCAACATTTC CGTGTGCGCC TTATTCCCTT TTTTGCGGCA TTTTGCCCTC CTGTTTTTGC  
3301 TCACCCAGAA ACGCTGGTGA AAGTAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG  
3361 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG  
3421 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGTTGA  
3481 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT AACTATTCT CAGAATGACT TGGTTGAGTA  
3541 CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC  
3601 TGCCATAACC ATGAGTGATA AACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC  
3661 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG  
3721 GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT GACACCACGA TGCCTGCAGC  
3781 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA  
3841 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT  
3901 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT  
3961 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG  
4021 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT  
4081 TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT  
4141 TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAT  
4201 CCCTTAACGT GAGTTTTTCG TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
4261 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
4321 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAAGTGG  
4381 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
4441 TGCTGCCAGT GGCAGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
4501 TAAGGCGCAG CGGTGCGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC  
4561 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCGA  
4621 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG  
4681 GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
4741 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
4801 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC  
4861 TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
4921 TCGCCGAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGAGCGCCT  
4981 GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAT ATGGTGCATC  
5041 CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGT ATACACTCCG CTATCGCTAC  
5101 GTGACTGGGT CATGGCTGCG CCCCAGACAC CGCCAACACC CGCTGACGCG CCCTGACGGG  
5161 CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT  
5221 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GCTGCGGTAA AGCTCATCAG  
5281 CGTGGTCGTG AAGCGATTCA CAGATGTCTG CCTGTTTATC CGCGTCCAGC TCGTTGAGTT  
5341 TCTCCAGAAG CGTTAATGTC TGGCTTCTGA TAAAGCGGGC CATGTTAAGG GCGGTTTTTT  
5401 CCTGTTTGGT CACTGATGCC TCCGTGTAAG GGGGATTTCT GTTCATGGGG GTAATGATAC  
5461 CGATGAAACG AGAGAGGATG CTCACGATAC GGGTTACTGA TGATGAACAT GCCCGGTTAC  
5521 TGGAACGTTG TGAGGGTAAA CAACTGGCGG TATGGATGCG GCGGGACCAG AGAAAAATCA  
5581 CTCAGGGTCA ATGCCAGCGC TTCGTTAATA CAGATGTAGG TGTTCCACAG GGTAGCCAGC  
5641 AGCATCCTGC GATGCAGATC CGGAACATAA TGGTGCAGGG CGCTGACTTC CGCGTTTCCA  
5701 GACTTTACGA AACACGGAAA CCGAAGACCA TTCATGTTGT TGCTCAGGTC GCAGACGTTT  
5761 TGCAGCAGCA GTCGCTTAC GTTCGCTCGC GTATCGGTGA TTCATTCTGC TAACCAGTAA  
5821 GGCAACCCCG CCAGCCTAGC CGGGTCCTCA ACGACAGGAG CACGATCATG CGCACCCGTG  
5881 GCCAGGACCC AACGCTGCCC GAGATGCGCC GCGTGCGGCT GCTGGAGATG GCGGACGCGA  
5941 TGGATATGTT CTGCCAAGGG TTGGTTTTCG CATTACAGT TCTCCGCAAG AATTGATTGG  
6001 CTCCAATTCT TGGAGTGGTG AATCCGTTAG CGAGGTGCCG CCGGCTTCCA TTCAGGTCGA  
6061 GGTGGCCCGG CTCCATGCAC CGCGACGCAA CGCGGGGAGG CAGACAAGGT ATAGGGCGGC

FIGURE 35C

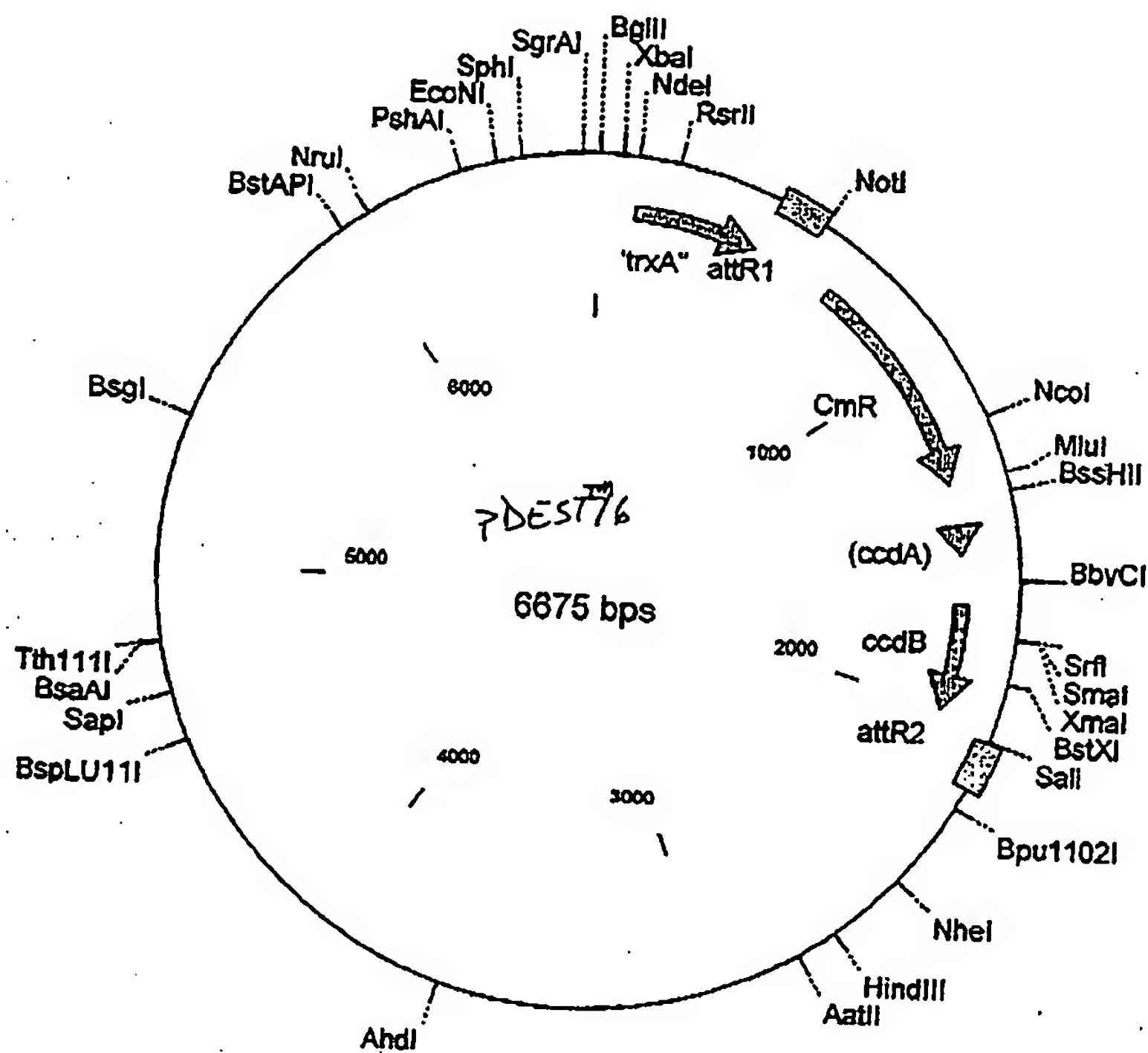
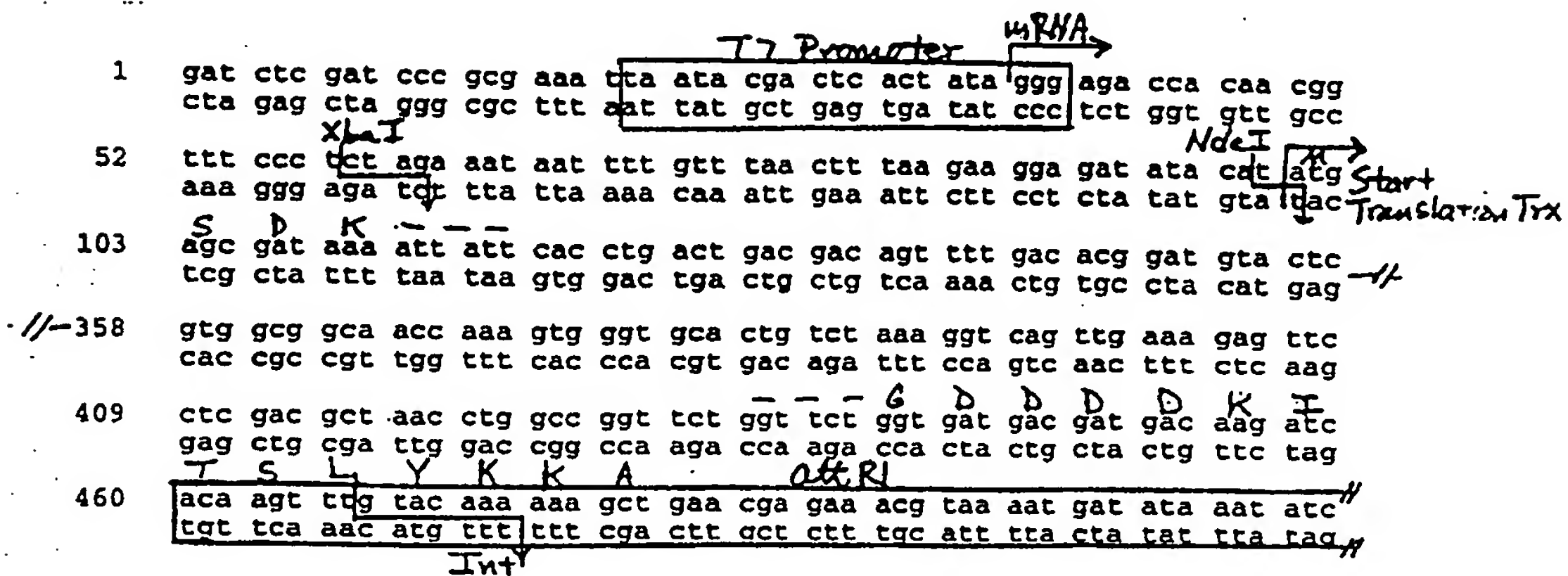
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6181 GCCTACAATC CATGCCAACC CGTTCCATGT GCTCGCCGAG GCGGCATAAA TCGCCGTGAC  
6241 GATCAGCGGT CCAGTGATCG AAGTTAGGCT GGTAAGAGCC GCGAGCGATC CTTGAAGCTG  
6301 TCCCTGATGG TCGTCATCTA CCTGCCTGGA CAGCATGGCC TGCAACGCGG GCATCCCGAT  
6361 GCCGCCGGAA GCGAGAAGAA TCATAATGGG GAAGGCCATC CAGCCTCGCG TCGCGAACGC  
6421 CAGCAAGACG TAGCCCAGCG CGTCGGCCGC CATGCCGGCG ATAATGGCCT GCTTCTCGCC  
6481 GAAACGTTTG GTGGCGGGAC CAGTGACGAA GGCTTGAGCG AGGGCGTGCA AGATTCCGAA  
6541 TACCGCAAGC GACAGGCCGA TCATCGTCGC GCTCCAGCGA AAGCGGTCCT CGCCGAAAA  
6601 GACCCAGAGC GCTGCCGGCA CCTGTCTTAC GAGTTGCATG ATAAAGAAGA CAGTCATAAG  
6661 TCGGGCGACG ATAGTCATGC CCCGCGCCCA CCGGAAGGAG CTGACTGGGT TGAAGGCTCT  
6721 CAAGGGCATC GGTCGATCGA CGCTCTCCCT TATGCGACTC CTGCATTAGG AAGCAGCCCA  
6781 GTAGTAGGTT GAGGCCGTTG AGCACC GCCG CCGCAAGGAA TGGTGCATGC AAGGAGATGG  
6841 CGCCCAACAG TCCCCCGGCC ACGGGGCCGTG CCACCATACC CACGCCGAAA CAAGCGCTCA  
6901 TGAGCCCGAA GTGGCGAGCC CGATCTTCCC CATCGGTGAT GTCGGCGATA TAGGCGCCAG  
6961 CAACCGCACC TGTGGCGCCG GTGATGCCGG CCACGATGCG TCCGGCGTAG AGG

FIGURE 351)

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**Figure 36A:**  $\gamma$ DEST16 Thioredoxin N-Fusion Protein  
in E. coli with T7 Promoter





## pDEST16 6675 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
104..457		trxA
585..461		attR1
694..1353		CmR
1473..1557		inactivated ccdA
1695..2000		ccdB
2041..2165		attR2

1	AGATCTCGAT	CCCGCGAAAT	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC
61	TAGAAATAAT	TTTGTTTAAC	TTTAAGAAGG	AGATATACAT	ATGAGCGATA	AAATTATTCA
121	CCTGACTGAC	GACAGTTTTG	ACACGGATGT	ACTCAAAGCG	GACGGGGCGA	TCCTCGTCGA
181	TTTCTGGGCA	GAGTGGTGCG	GTCCGTGCAA	AATGATCGCC	CCGATTCTGG	ATGAAATCGC
241	TGACGAATAT	CAGGGCAAAC	TGACCGTTGC	AAAACCTGAAC	ATCGATCAAA	ACCCTGGCAC
301	TGCGCCGAAA	TATGGCATCC	GTGGTATCCC	GACTCTGCTG	CTGTTCAAAA	ACGGTGAAGT
361	GGCGGCAACC	AAAGTGGGTG	CACTGTCTAA	AGGTCAGTTG	AAAGAGTTCC	TCGACGCTAA
421	CCTGGCCGGT	TCTGGTTCTG	GTGATGACGA	TGACAAGATC	ACAAGTTTGT	ACAAAAAAGC
481	TGAACGAGAA	ACGTAAAATG	ATATAAATAT	CAATATATTA	AATTAGATTT	TGCATAAAAA
541	ACAGACTACA	TAATACTGTA	AAACACAACA	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC
601	ACCCGAGGCT	TTACACTTTA	TGCTTCCGGC	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT
661	CCGGCGAGAT	TTTCAGGAGC	TAAGGAAGCT	AAAATGGAGA	AAAAAATCAC	TGGATATACC
721	ACCGTTGATA	TATCCCAATG	GCATCGTAAA	GAACATTTTG	AGGCATTTCA	GTCAGTTGCT
781	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG	GATATTACGG	CCTTTTAA	GACCGTAAAG
841	AAAAATAAGC	ACAAGTTTTA	TCCGGCCTTT	ATTCACATTC	TTGCCCGCCT	GATGAATGCT
901	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC	GGTGAGCTGG	TGATATGGGA	TAGTGTTCAC
961	CCTTGTTACA	CCGTTTTCCA	TGAGCAAAC	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC
1021	CACGACGATT	TCCGGCAGTT	TCTACACATA	TATTTCGCAAG	ATGTGGCGTG	TTACGGTGAA
1081	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC
1141	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC	GTGGCCAATA	TGGACAACCT	CTTCGCCCCC
1201	GTTTTACCA	TGGGCAAATA	TTATACGCAA	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT
1261	CAGGTTTCATC	ATGCCGTCTG	TGATGGCTTC	CATGTCGGCA	GAATGCTTAA	TGAATTACAA
1321	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG	TAAACGCGTG	GATCCGGCTT	ACTAAAAGCC
1381	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT	TTTTGCGGTA	TAAGAATATA	TACTGATATG
1441	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG	CTATGAAGCA	GCGTATTACA	GTGACAGTTG
1501	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT	ATATGATGTC	AATATCTCCG	GTCTGGTAAG
1561	CACAACCATG	CAGAATGAAG	CCCGTCGTCT	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA
1621	GGAAGGGATG	GCTGAGGTG	CCCGGTTTAT	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA
1681	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT	ACACCTATAA	AAGAGAGAGC	CGTTATCGTC
1741	TGTTTGTGGA	TGTACAGAGT	GATATTATTG	ACACGCCCCG	GCGACGGATG	GTGATCCCCC
1801	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG	TCTCCCGTGA	ACTTTACCCG	GTGGTGCATA
1861	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA
1921	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC	GCGAAAATGA	CATCAAAAAC	GCCATTAAAC
1981	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT	CCCTTATACA	CAGCCAGTCT	GCAGGTCGAC
2041	CATAGTGACT	GGATATGTTG	TGTTTTACAG	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC
2101	TAATTTAATA	TATTGATATT	TATATCATTT	TACGTTTCTC	GTTTCTGCTT	CTTGTACAAA
2161	GTGGTGATGA	TCCGGCTGCT	AACAAAGCCC	GAAAGGAAGC	TGAGTTGGCT	GCTGCCACCG
2221	CTGAGCAATA	ACTAGCATAA	CCCCTTGGGG	CCTCTAAACG	GGTCTTGAGG	GGTTTTTTTG
2281	TGAAAGGAGG	AACTATATCC	GGATATCCAC	AGGACGGGTG	TGGTCGCCAT	GATCGCGTAG
2341	TCGATAGTGG	CTCCAAGTAG	CGAAGCGAGC	AGGACTGGGC	GGCGGCCAAA	GCGGTCCGAC
2401	AGTGCTCCGA	GAACGGGTGC	GCATAGAAAT	TGCATCAACG	CATATAGCGC	TAGCAGCAGC
2461	CCATAGTGAC	TGGCGATGCT	GTCGGAATGG	ACGATATCCC	GCAAGAGGCC	CGGCAGTACC
2521	GGCATAACCA	AGCCTATGCC	TACAGCATCC	AGGGTGACGG	TGCCGAGGAT	GACGATGAGC
2581	GCATTGTTAG	ATTTTCATACA	CGGTGCCTGA	CTGCGTTAGC	AATTTAACTG	TGATAAACTA
2641	CCGCATTAAA	GCTTATCGAT	GATAAGCTGT	CAAACATGAG	AATTCTTGAA	GACGAAAGGG
2701	CCTCGTGATA	CGCCTATTTT	TATAGGTTAA	TGTCATGATA	ATAATGGTTT	CTTAGACGTC
2761	AGGTGGCACT	TTTCGGGGAA	ATGTGCGCGG	AACCCCTATT	TGTTTATTTT	TCTAAATACA-

Figure 36B

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2821 TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA  
2881 AAGGAAGAGT ATGAGTATTC AACATTTCCTG TGTGCGCCCTT ATTCCTTTT TTGCGGCATT  
2941 TTGCCTTCCT GTTTTGTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA  
3001 GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG  
3061 TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC TATGTGGCGC  
3121 GGTATTATCC CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA  
3181 GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT  
3241 AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT  
3301 GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT  
3361 AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA  
3421 CACCACGATG CCTGCAGCAA TGGCAACAAC GTTGCAGCAA CTATTAAGTG GCGAACTACT  
3481 TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC  
3541 ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG GAGCCGGTGA  
3601 GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT  
3661 AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA  
3721 GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT  
3781 TTAGATTGAT TTAAAACTTC ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTGA  
3841 TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT  
3901 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTCTG CGCGTAATCT GCTGCTTGCA  
3961 AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT  
4021 TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA  
4081 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT  
4141 AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGAAGTC  
4201 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACAC  
4261 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA  
4321 AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG  
4381 AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT  
4441 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG  
4501 CCTATGGAAA AACGCCAGCA ACGCGGCCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT  
4561 TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT  
4621 TGAGTGAGCT GATACCGCTC GCCGCGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA  
4681 GGAAGCGGAA GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA  
4741 CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT  
4801 ACACCTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG  
4861 CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG  
4921 TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC  
4981 TGCGGTAAAG CTCATCAGCG TGGTCGTGAA GCGATTCAAC GATGTCTGCC TGTTCATCCG  
5041 CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA  
5101 TGTTAAGGGC GGTTTTTTCC TGTGTTGCTA CTGATGCCTC CGTGTAAAGG GGATTTCTGT  
5161 TCATGGGGGT AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACCG GTTACTGATG  
5221 ATGAACATGC CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC  
5281 GGGACCAGAG AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG  
5341 TTCCACAGGG TAGCCAGCAG CATCCTGCGA TGCAGATCCG GAACATAATG GTGCAGGGCG  
5401 CTGACTTCCG CGTTTCCAGA CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG  
5461 CTCAGGTCCG AGACGTTTTG CAGCAGCAGT CGCTTCACGT TCGCTCGCGT ATCGGTGATT  
5521 CATTCTGCTA ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCTCAAC GACAGGAGCA  
5581 CGATCATGCG CACCCGTGGC CAGGACCCAA CGCTGCCCGA GATGCGCCGC GTGCGGCTGC  
5641 TGGAGATGGC GGACGCGATG GATATGTTCT GCCAAGGGTT GGTGTCGCA TTCACAGTTC  
5701 TCCGCAAGAA TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC  
5761 GGCTTCCATT CAGGTGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CCGGGAGGCA  
5821 GACAAGGTAT AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC  
5881 GGCATAAATC GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC  
5941 GAGCGATCCT TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG  
6001 CAACGCGGGC ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA  
6061 GCCTCGCGTC GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCA TGCCGGCGAT  
6121 AATGGCCTGC TTCTCGCCGA AACGTTTGGT GGCGGGACCA GTGACGAAGG CTTGAGCGAG  
6181 GGCGTGCAAG ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA  
6241 GCGGTCCCTG CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCTACGA GTTGCATGAT

FIGURE 36C

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6301 AAAGAAGACA GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT  
6361 GACTGGGTTG AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TCGACTCCT  
6421 GCATTAGGAA GCAGCCCAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG  
6481 GTGCATGCAA GGAGATGGCG CCCAACAGTC CCCC GGCCAC GGGGCCTGCC ACCATACCCA  
6541 CGCCGAAACA AGCGCTCATG AGCCCGAAGT GGCGAGCCCG ATCTTCCCA TCGGTGATGT  
6601 CGGCGATATA GGCGCCAGCA ACCGCACCTG TGGCGCCGGT GATGCCGGCC ACGATGCGTC  
6661 CGGCGTAGAG GATCG

FIGURE 36D

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T7 Promoter      mRNA

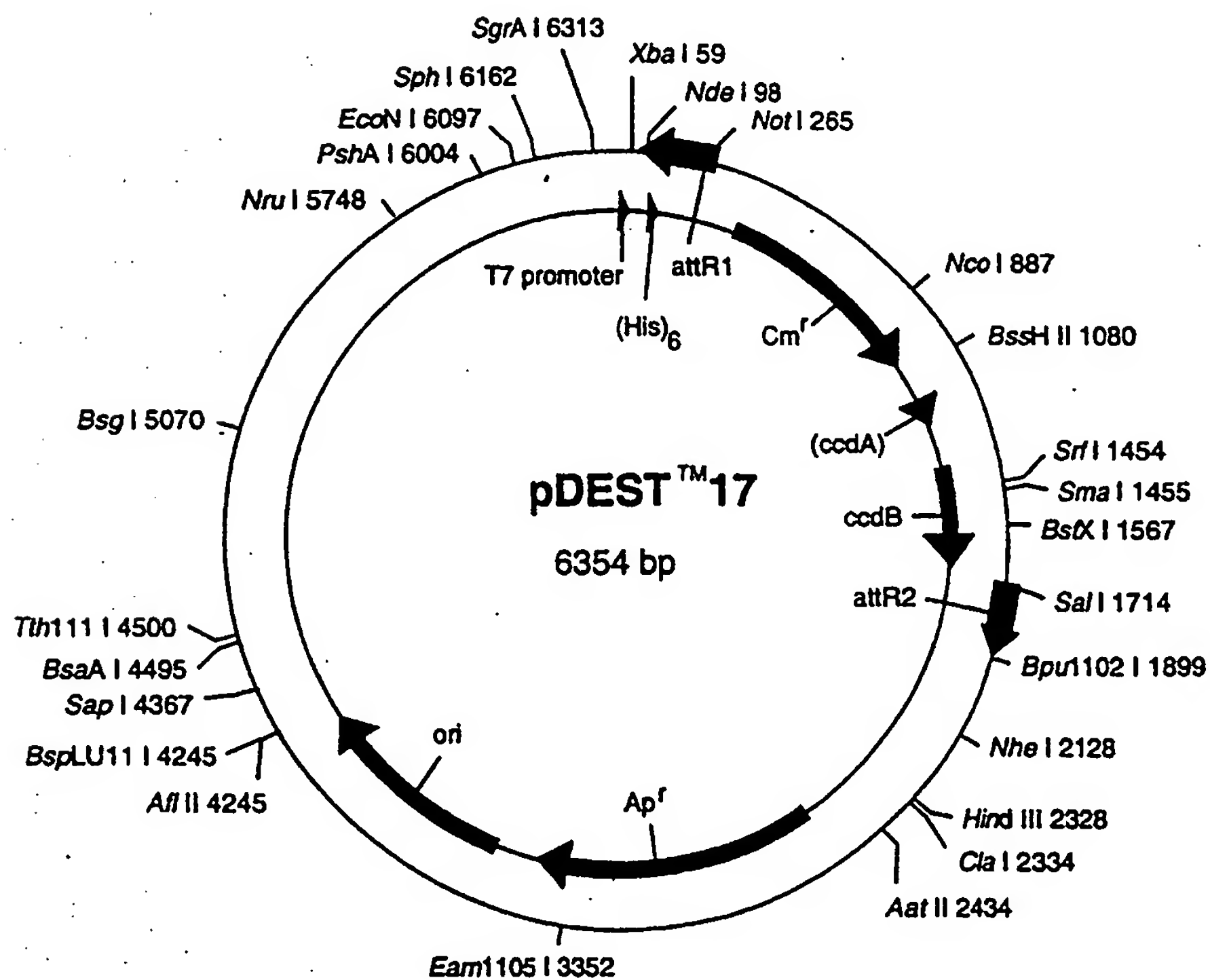
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1  gat ccc gcg aaa tta ata cga ctc act ata ggg aga cca caa cgg ttt ccc
   cta ggg cgc ttt aat tat gct gag tga tat ccc tct ggt gtt gcc aaa ggg

52  tct aga aat aat ttt gtt taa ctt taa gaa gga gat ata cat atg tgg tac
   aga tct tta tta aaa caa att gaa att ctt cct cta tat gta tac agc atg

103 Y H H H H H H L E S T S L Y K K A //
    tac cat cac cat cac cat cac ctc gaa tca aca agt tgg tac aaa aaa gct //
    atg gta gtg gta gtg gta gtg gag ctt agt tgt tca aac atg ttt ttt cga //
                                     attR1      Int
  
```

Start Translation



## pDEST17 6354 bp

Location (Base Nos.)	Gene Encoded
258..134	attR1
367..1026	CmR
1146..1230	inactivated ccdA
1368..1673	ccdB
1714..1838	attR2
2564..3421	ampR

```
1 CGATCCCGCG AAATTAATAC GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGAAA
61 TAATTTTGTT TAACTTTAAG AAGGAGATAT ACATATGTCG TACTACCATC ACCATCACCA
121 TCACCTCGAA TCAACAAGTT TGTACAAAAA AGCTGAACGA GAAACGTAAA ATGATATAAA
181 TATCAATATA TTAAATTAGA TTTTGCATAA AAAACAGACT ACATAATACT GTAAAAACACA
241 ACATATCCAG TCACTATGGC GGCCGCATTA GGCACCCAG GCTTTACACT TTATGCTTCC
301 GGCTCGTATA ATGTGTGGAT TTTGAGTTAG GATCCGTCGA GATTTTCAGG AGCTAAGGAA
361 GCTAAAATGG AGAAAAAAT CACTGGATAT ACCACCGTTG ATATATCCCA ATGGCATCGT
421 AAAGAACATT TTGAGGCATT TCAGTCAGTT GCTCAATGTA CCTATAACCA GACCGTTCAG
481 CTGGATATTA CGGCCTTTT AAAGACCGTA AAGAAAAATA AGCACAAGTT TTATCCGGCC
541 TTTATTCACA TTCTTGCCCG CCTGATGAAT GCTCATCCGG AATTCCGTAT GGCAATGAAA
601 GACGGTGAGC TGGTGATATG GGATAGTGTT CACCCTTGTT ACACCGTTT CCATGAGCAA
661 ACTGAAACGT TTTTCATCGCT CTGGAGTGAA TACCACGACG ATTTCCGGCA GTTTCTACAC
721 ATATATTTCG AAGATGTGGC GTGTTACGGT GAAAACCTGG CCTATTTCCC TAAAGGGTTT
781 ATTGAGAATA TGTTTTTCGT CTCAGCCAAT CCCTGGGTGA GTTTCACCAG TTTTGATTTA
841 AACGTGGCCA ATATGGACAA CTTCTTCGCC CCCGTTTTCA CCATGGGCAA ATATTATACG
901 CAAGGCGACA AGGTGCTGAT GCCGCTGGCG ATTCAGGTTT ATCATGCCGT CTGTGATGGC
961 TTCCATGTCG GCAGAATGCT TAATGAATTA CAACAGTACT GCGATGAGTG GCAGGGCGGG
1021 GCGTAAAGAT CTGGATCCCG CTTACTAAAA GCCAGATAAC AGTATGCGTA TTTGCGCGCT
1081 GATTTTTGCG GTATAAGAAT ATATACTGAT ATGTATACCC GAAGTATGTC AAAAAGAGGT
1141 GTGCTATGAA GCAGCGTATT ACAGTGACAG TTGACAGCGA CAGCTATCAG TTGCTCAAGG
1201 CATATATGAT GTCAATATCT CCGGTCTGGT AAGCACAACC ATGCAGAATG AAGCCCGTCG
1261 TCTGCGTGCC GAACGCTGGA AAGCGGAAAA TCAGGAAGGG ATGGCTGAGG TCGCCCGGTT
1321 TATTGAAATG AACGGCTCTT TTGCTGACGA GAACAGGGAC TGGTGAAATG CAGTTTAAGG
1381 TTTACACCTA TAAAAGAGAG AGCCGTTATC GTCTGTTTGT GGATGTACAG AGTGATATTA
1441 TTGACACGCC CGGGCGACGG ATGGTGATCC CCCTGGCCAG TGCACGCTG CTGTCAGATA
1501 AAGTCTCCCG TGAACCTTAC CCGGTGGTGC ATATCGGGGA TGAAAGCTGG CGCATGATGA
1561 CCACCGATAT GGCCAGTGTG CCGGTCTCCG TTATCGGGGA AGAAGTGGCT GATCTCAGCC
1621 ACCGCGAAAA TGACATCAAA AACGCCATTA ACCTGATGTT CTGGGGAATA TAAATGTCAG
1681 GCTCCCTTAT ACACAGCCAG TCTGCAGGTC GACCATAGTG ACTGGATATG TTGTGTTTTA
1741 CAGTATTATG TAGTCTGTTT TTTATGCAAA ATCTAATTTA ATATATTGAT ATTTATATCA
1801 TTTTACGTTT CTCGTTACAG TTTCTTGATC AAAGTGGTTG ATTCGAGGCT GCTAACAAAG
1861 CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAAC TAGCA TAACCCCTTG
1921 GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACTATA TCCGGATATC
1981 CACAGGACGG GTGTGGTCGC CATGATCGCG TAGTCGATAG TGGCTCCAAG TAGCGAAGCG
2041 AGCAGGACTG GGCGGCGGCC AAAGCGGTCG GACAGTGCTC CGAGAACGGG TCGGCATAGA
2101 AATTGCATCA ACGCATATAG CGCTAGCAGC ACGCCATAGT GACTGGCGAT GCTGTCGGAA
2161 TGGACGATAT CCCGCAAGAG GCCCGGCAGT ACCGGCATAA CCAAGCCTAT GCCTACAGCA
2221 TCCAGGGTGA CCGTGCCGAG GATGACGATG AGCGCATTGT TAGATTTTCAT ACACGGTGCC
2281 TGA CTGCGTT AGCAATTTAA CTGTGATAAA CTACCGCATT AAAGCTTATC GATGATAAGC
2341 TGTCAAACAT GAGAATCTT GAAGACGAAA GGGCCTCGTG ATACGCCTAT TTTTATAGGT
2401 TAATGTCATG ATAATAATGG TTTCTTAGAC GTCAGGTGGC ACTTTTCGGG GAAATGTGCG
2461 CGGAACCCCT ATTTGTTTAT TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA
2521 ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT
2581 CCGTGTCGCC CTTATTCCCT TTTTTCGGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA
2641 AACGCTGGTG AAAGTAAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG GTTACATCGA-
```

FIGURE 37B



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2701 ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAC GTTTTCCAAT  
2761 GATGAGCACT TTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG ACGCCGGGCA  
2821 AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT  
2881 CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC  
2941 CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT  
3001 AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACCTCGC CTTGATCGTT GGAACCGGA  
3061 GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG CAATGGCAAC  
3121 AACGTTGCGC AAACCTATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAAATTAAT  
3181 AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC TTCCGGCTGG  
3241 CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA TCATTGCAGC  
3301 ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC  
3361 AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG  
3421 GTAACGTGCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTAA  
3481 ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG  
3541 TGAGTTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT CTTCTTGAGA  
3601 TCCTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC TACCAGCGGT  
3661 GGTTTGTTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAAGT GCTTCAGCAG  
3721 AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC ACTTCAAGAA  
3781 CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG CTGCTGCCAG  
3841 TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG ATAAGGCGCA  
3901 GCGGTCGGGC TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA CGACCTACAC  
3961 CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG AAGGGAGAAA  
4021 GGCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA GGGAGCTTCC  
4081 AGGGGGAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT GACTTGAGCG  
4141 TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAACGCCA GCAACGCGGC  
4201 CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC CTGCGTTATC  
4261 CCCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG CTCGCCGAG  
4321 CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC TGATGCGGTA  
4381 TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC TCTCAGTACA  
4441 ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA CGTGACTGGG  
4501 TCATGGCTGC GCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG GCTTGTCTGC  
4561 TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTGAGAGGT  
4621 TTTCACCGTC ATCACCGAAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA GCGTGGTCGT  
4681 GAAGCGATTG ACAGATGTCT GCCTGTTTCT CCGCGTCCAG CTCGTTGAGT TTCTCCAGAA  
4741 GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGTTTTTT TCCTGTTTGG  
4801 TCACTGATGC CTCCGTGTAA GGGGGATTTC TGTTTCATGGG GGTAAATGATA CCGATGAAAC  
4861 GAGAGAGGAT GCTCACGATA CGGGTTACTG ATGATGAACA TGCCCGGTTA CTGGAACGTT  
4921 GTGAGGGTAA ACAACTGGCG GTATGGATGC GGCGGGACCA GAGAAAAATC ACTCAGGGTC  
4981 AATGCCAGCG CTTGCTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG CAGCATCCTG  
5041 CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTCC AGACTTTACG  
5101 AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT TTGCAGCAGC  
5161 AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCATTCTG CTAACCAGTA AGGCAACCCC  
5221 GCCAGCCTAG CCGGGTCTCT AACGACAGGA GCACGATCAT GCGCACCCGT GGCCAGGACC  
5281 CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GCGGACGCG ATGGATATGT  
5341 TCTGCCAAGG GTTGGTTTGC GCATTCACAG TTCTCCGCAA GAATTGATTG GCTCCAATTC  
5401 TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCCGGCTTCC ATTCAGGTCG AGGTGGCCCCG  
5461 GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG CGCCTACAAT  
5521 CCATGCCAAC CCGTTCCATG TGCTCGCCGA GGCGGCATAA ATCGCCGTGA CGATCAGCGG  
5581 TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT GTCCCTGATG  
5641 GTCGTCTCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA TGCCGCCGGA  
5701 AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAACC CCAGCAAGAC  
5761 GTAGCCCAGC GCGTCGGCCG CCATGCCGGC GATAATGGCC TGCTTCTCGC CGAAACGTTT  
5821 GGTGGCGGGA CCACTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA ATACCGCAAG  
5881 CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA TGACCCAGAG  
5941 CGCTGCCGGC ACCTGTCCTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA GTGCGGCGAC  
6001 GATAGTCATG CCCCAGCGCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC TCAAGGGCAT  
6061 CGGTGATCG ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC AGTAGTAGGT  
6121 TGAGGCCGTT GAGCACCAGC GCCGCAAGGA ATGGTGCATG CAAGGAGATG GCGCCCAACA-

Figure 37C



6181 GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC ATGAGCCCGA  
6241 AGTGGCGAGC CCGATCTTCC CCATCGGTGA TGTCGGCGAT ATAGGCGCCA GCAACCGCAC  
6301 CTGTGGCGCC GGTGATGCCG GCCACGATGC GTCCGGCGTA GAGGATCGAG ATCT

FIGURE 37D

Figure 38A: pDEST18

FastBac Transfer Vector with p10  
Baculovirus Promoter

1 gaagacctcg gcegtcgagg cgcttgccgg tgggtgetgac cccggatgaa gtgggttcgca  
cttctggagc cggcagcgcc gcgaacggcc accacgactg gggcctactt caccaagcgt

61 tectcggttt tctggaagge gagcatcggt tgttegecca ggactctage tatagtteta  
aggagccaaa agaccttccg ctcgtagcaa acaagcgggt cctgagatcg atatcaagat

121 gtgggttggt acgtatcgag caagaaata anacggcaca cgcgtggag tcttgtgtgc  
caccaaccga tgcatagtct gttcttttat tttgctgtt gcgaacctc agaacacacg

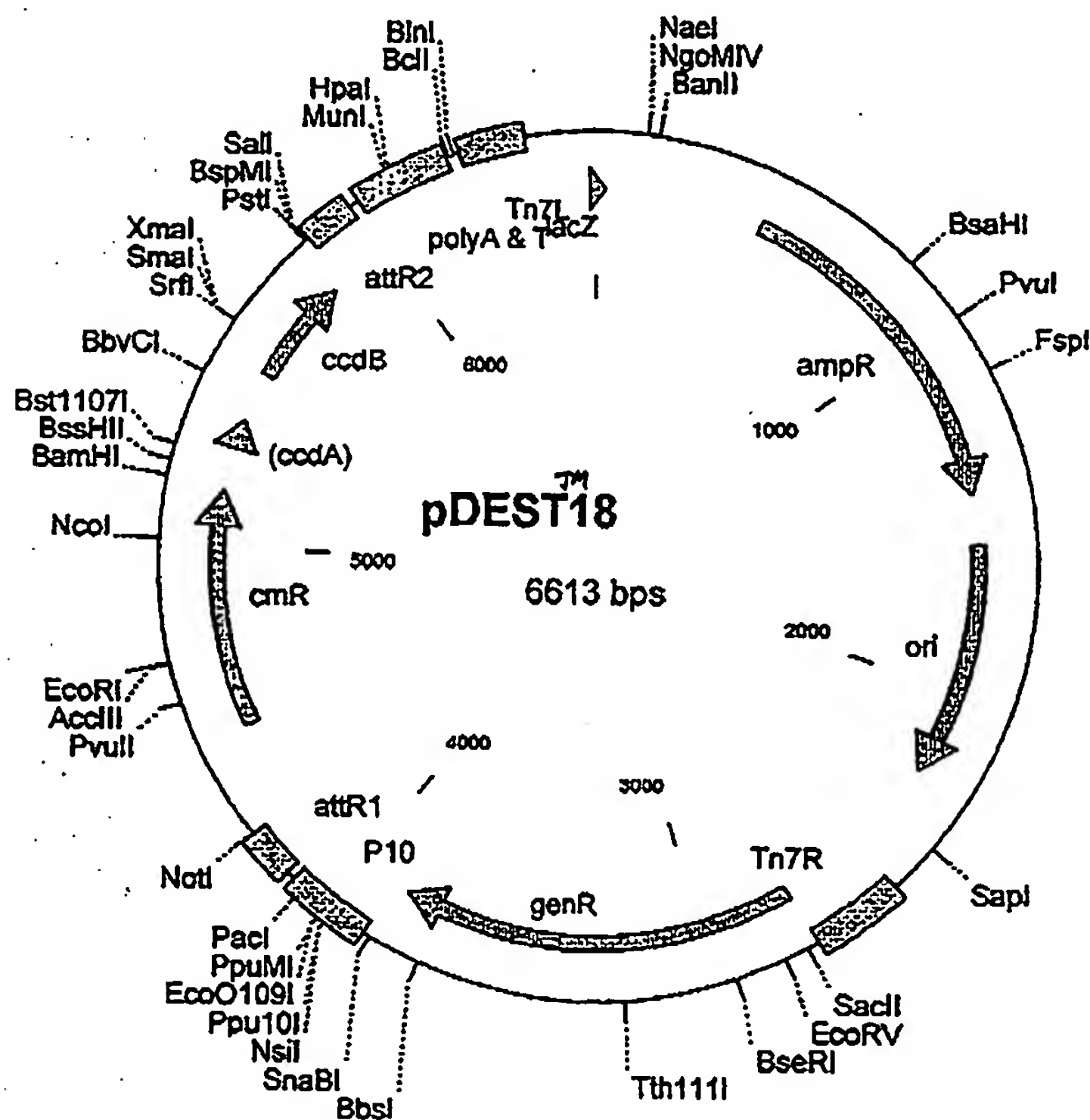
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//ataaaaatgt ttctaagtct ttatgcgtag tgaatgtgt tccccctgat accttaatac//

241 //gatcttgagg atgcccggag ctttaatca acccaacaca atatattata gttaaatagg// mRNA  
//ataaaactcc tacggccctg gaaattaat tgggtgtgt tatataatat caatttatc//

301 //aattatctat caaatcatt gtatatataat taaaatacta tactgtaaat tacattttat  
//taataaata gtttagtata oataataatta attttatgat atgacattta atgtaaaata

361 ttacaatgag gatcatcaca agtttgtaca aaaaagctga acgagaaacg taaaatgata  
aatgttactc ctagtagtgt tcaaacatgt ttttgcact tgcctttgc attttactat//

Int. attR1



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## pDEST18 6613 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
474..1449	ampR
1590..2244	ori
2738..3850	genR
4251..4127	attR1
4501..5160	CmR
5280..5364	inactivated ccdA
5502..5807	ccdB
5848..5972	attR2
6595..25	lacZ

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1 GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC
61 GCTACACTTG CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC
121 ACGTTCGCCG GCTTTCCTCC TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT
181 AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG
241 CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT
301 GGACTCTTGT TCCAAACTGG AACAACTC AACCTATCT CGGTCTATTC TTTTGATTTA
361 TAAGGGATTT TGCCGATTTT GGCCTATTGG TTAATAAATG AGCTGATTTA ACAAAAATTT
421 AACGCGAATT TTAACAAAT ATTAACGTTT ACAATTTTCA GTGGCACTTT TCGGGGAAAT
481 GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG
541 AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA
601 CATTTCCTTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC
661 CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTC
721 ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT
781 CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC
841 GGGCAAGAGC AACTCGGTCT CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA
901 CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC
961 ATAACCATGA GTGATAACAC TCGCGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG
1021 GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA
1081 CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG
1141 GCAACAACGT TGCGCAAAC ATTAAGTGGC GAACACTTAA CTCTAGCTTC CCGGCAACAA
1201 TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG
1261 GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CGGTATCATT
1321 GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT
1381 CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAA
1441 CATTGGTAAC TGTGAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT
1501 TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT
1561 TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT
1621 TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAA CAAAAAACC ACCGCTACCA
1681 GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC
1741 AGCAGAGCGC AGATACCAA TACTGTCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC
1801 AAGAACTCTG TAGCACCACC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT
1861 GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG
1921 GCGCAGCGGT CGGGCTGAAC GGGGGGTTCC TGCACACAGC CCAGCTTGA GCGAACGACC
1981 TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG
2041 AGAAAGGCGG ACAGGTATCC GGTAAAGCGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG
2101 CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT
2161 GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC
2221 GCGGCCTTTT TACGGTTTCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG
2281 TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC
2341 CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG
2401 CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACAC GCAGACCAGC CGCGTAACCT
2461 GGCAAAATCG GTTACGGTTG AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA-
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FIGURE 38B

2521 CAATAAAGTC TTAAACTGAA CAAATAGAT CTAAACTATG ACAATAAAGT CTAAACTAG  
2581 ACAGAATAGT TGTAAGCTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAE ACTGGACTTT  
2641 TGTTATGGCT AAAGCAAACCT CTTCAATTTT TGAAGTGCAA ATTGCCCGTC STATTAAAGA  
2701 GGGGCGTGGC CAAGGGCATG GTAAAGACTA TATTCGCGGC GTTGTGACAA TTTACCGAAC  
2761 AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GTACTTGGG  
2821 TCGATATCAA AGTGCATCAC TTCTTCCCCT ATGCCCAACT TTGTATAGAG AGCCACTGCG  
2881 GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA  
2941 TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGA3ACT  
3001 GCGAGATCAT AGATATAGAT CTCATACTGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC  
3061 GCGAGAGCGC CAACAACCGC TTCTTGCTCG AAGGCAGCAA GCGCGATGAA TGTCTTACTA  
3121 CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTAC3TCT  
3181 CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGG3CCG  
3241 AGCCTACATG TGCGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG  
3301 CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA  
3361 TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCGA GGCATAGACT GTACAA3AAA  
3421 ACAGTCATAA CAAGCCATGA AAACCGCCAC TCGCGCGTTA CCACCGCTGC GTTCGGTCAA  
3481 GGTTCTGGAC CAGTTGCGTG AGCGCATACG CTACTTGCA TACAGTTTAC GAACCG3ACA  
3541 GGCTTATGTC AACTGGGTTC GTGCCCTTCT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC  
3601 CTTGGGCAGC AGCGAAGTCG AGGCATTTCT GTCCTGGCTG GCGAACGAGC GCAAGGTTTC  
3661 GGTCTCCACG CATCGTCAGG CATTGGCGGC CTTGCTGTTT TTCTACGGCA AGGTGCTGTG  
3721 CACGATCTG CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGC3GGT  
3781 GGTGCTGACC CCGGATGAAG TGGTTCGCAT CCTCGGTTTT CTGGAAGGCG AGCATC3TTT  
3841 GTTCGCCCAG GACTCTAGCT ATAGTTCTAG TGGTTGGCTA CGTATCGAGC AAGAAAATAA  
3901 AACGCCAAAC GCGTTGGAGT CTTGTGTGCT ATTTTACAA AGATTCAAGAA ATACGCATCA  
3961 CTTACAACAA GGGGGACTAT GAAATTATGC ATTTTGAGGA TGCCGGGACC TTTAATTCAA  
4021 CCCAACACAA TATATTATAG TTAAATAAGA ATTATTTATC AAATCATTTG TATATTAAAT  
4081 AAAATACTAT ACTGTAAATT ACATTTTATT TACAATGAGG ATCATCACAA GTTTGTACAA  
4141 AAAAGCTGAA CGAGAAACGT AAAATGATAT AAATATCAAT ATATTAAAT AGATTTTGCA  
4201 TAAAAAACAG ACTACATAAT ACTGTAAAAC ACAACATATC CAGTCACTAT GGCGGC3GCT  
4261 AAGTTGGCAG CATCACCCGA CGCACTTTGC GCCGAATAAA TACCTGTGAC GGAAGATCAC  
4321 TTCGCAGAAT AAATAAATCC TGGTGTCCCT GTTGATACCG GGAAGCCCTG GGCCAACTTT  
4381 TGGCGAAAAT GAGACGTTGA TCGGCACGTA AGAGGTTCCA ACTTTCACCA TAATGAAATA  
4441 AGATCACTAC CGGGCGTATT TTTTGAGTTA TCGAGATTTT CAGGAGCTAA GGAAGCTAAA  
4501 ATGGAGAAAA AAATCACTGG ATATACCACC GTTGATATAT CCAATGGCA TCGTAAAGAA  
4561 CATTTTGAGG CATTTAGTC AGTTGCTCAA TGTACCTATA ACCAGACCGT TCAGCT3GAT  
4621 ATTACGGCCT TTTTAAAGAC CGTAAAGAAA AATAAGCACA AGTTTATCC GGCCTTTATT  
4681 CACATTCCTG CCCGCTGAT GAATGCTCAT CCGGAATTCC GTATGGCAAT GAAAGACGGT  
4741 GAGCTGGTGA TATGGGATAG TGTTCACCCT TGTTACACCG TTTTCCATGA GCAAAC3GAA  
4801 ACGTTTTCTAT CGCTCTGGAG TGAATACCAC GACGATTTCC GGCAGTTTCT ACACATATAT  
4861 TCGCAAGATG TGGCGTGTTA CGGTGA3AAC CTGGCCTATT TCCCTAAAGG GTTTATTGAG  
4921 AATATGTTTT TCGTCTCAGC CAATCCCTGG GTGAGTTTCA CCAGTTTGA TTTAAAC3GTG  
4981 GCCAATATGG ACAACTTCTT CGCCCCCGTT TTCACCATGG GCAAATATTA TACGCAAGGC  
5041 GACAAGGTGC TGATGCCGCT GCGGATTCAG GTTCATCATG CCGTCTGTGA TGGCTTCCAT  
5101 GTCGGCAGAA TGCTTAATGA ATTACAACAG TACTGCGATG AGTGGCAGGG CGGGGC3TAA  
5161 ACGCGTGGAT CCGGCTTACT AAAAGCCAGA TAACAGTATG CGTATTTGCG CGCTGA3TTT  
5221 TGCGGTATAA GAATATATAC TGATATGTAT ACCCGAAGTA TGTCAAAAAG AGGTGT3CTA  
5281 TGAAGCAGCG TATTACAGTG ACAGTTGACA GCGACAGCTA TCAGTTGCTC AAGGCATATA  
5341 TGATGTCAAT ATCTCCGGTC TGGTAAGCAC AACCATGCAG AATGAAGCCC CTCGTCTGCG  
5401 TGCCGAACGC TGGAAAGCGG AAAATCAGGA AGGGATGGCT GAGGTCGCCC GGTTTATTGA  
5461 AATGAACGGC TCTTTTGCTG ACGAGAACAG GGACTGGTGA AATGCAGTTT AAGGTTTACA  
5521 CCTATAAAAG AGAGAGCCGT TATCGTCTGT TTGTGGATGT ACAGAGTGAT ATTATT3ACA  
5581 CGCCCGGGCG ACGGATGGTG ATCCCCCTGG CCAGTGCACG TCTGCTGTCA GATAAA3TCT  
5641 CCCGTGAACT TTACCCGGTG GTGCATATCG GGGATGAAAG CTGGCGCATG ATGACCACCG  
5701 ATATGGCCAG TGTGCCGGTC TCCGTTATCG GGGGAAGAAG GGCTGATCTC AGCCAC3CGG  
5761 AAAATGACAT CAAAACGCC ATTAACCTGA TGTCTGGGG AATATAAATG TCAGGCT3CC  
5821 TTATACACAG CCAGTCTGCA GGTCGACCAT AGTGACTGGA TATGTTGTGT TTTACAGTAT  
5881 TATGTAGTCT GTTTTTTATG CAAAATCTAA TTTAATATAT TGATATTTAT ATCATTTTAC  
5941 GTTCTCGTT CAGCTTTCTT GTACAAAGTG GTGATAGCTT GTCGAGAAGT ACTAGAGGAT-

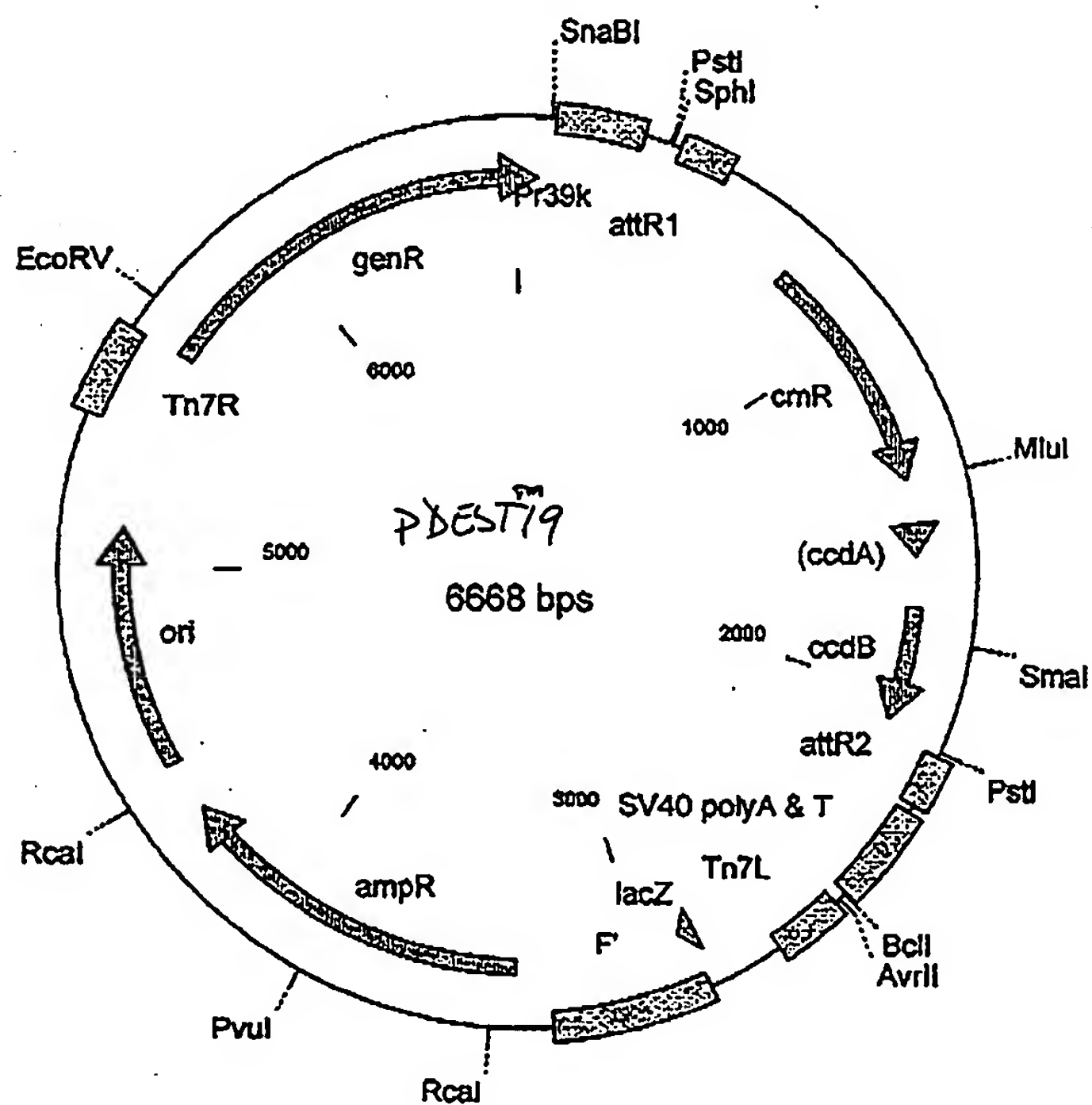
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6001 CATAATCAGC CATACCACAT TTGTAGAGGT TTTACTTGCT TTAAAAAACC TCCCACACCT  
6061 CCCCTGAAC CTGAAACATA AAATGAATGC AATTGTTGTT GTTAACTTGT TTATTGCAGC  
6121 TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTTC ACAAATAAAG CATTTTTTTC  
6181 ACTGCATTCT AGTTGTGGTT TGTCCAAACT CATCAATGTA TCTTATCATG TCTGGATCTG  
6241 ATCACTGCTT GAGCCTAGGA GATCCGAACC AGATAAGTGA AATCTAGTTC CAAACTATTT  
6301 TGTCATTTTT AATTTTCGTA TTAGCTTACG ACGCTACACC CAGTTCCCAT CTATTTTGTC  
6361 ACTCTTCCCT AAATAATCCT TAAAAACTCC ATTTCCACCC CTCCCAGTTC CCAACTATTT  
6421 TGTCCGCCCA CAGCGGGGCA TTTTCTTCC TGTATGTTT TTAATCAAAC ATCCTGCCAA  
6481 CTCCATGTGA CAAACCGTCA TCTTCGGCTA CTTTTCTCT GTCACAGAAT GAAAATTTTT  
6541 CTGTCATCTC TTCGTTATTA ATGTTTGTA TTAGCTGAAT ATCAACGCTT ATTTGCAGCC  
6601 TGAATGGCGA ATG

FIGURE 38D

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1 ggtgacgccc tcatttttcc attgtaacgt aaatggcaac ttgtagatga acgcgctgtc  
 ccactgcggc agtagaaagg taacattgca tttaccgttg aacatctact tgcgcgacag  
 61 aaaaaaccgg ccagtttctt ccacaaactc gcgcacggct gtctcgtaaa cttttgcgtc  
 ttttttgccc ggtcaaagaa ggtgtttgag cgcgtgcega cagagcattt gaaaacgcag  
 121 // gcaacaatcg cgatgacctc gtggtatgga aatttttctt aaaaaagtgt cgttcatgtc  
 // cgttgttagc gctactggag caccatacct ttaaaaaaga ttttttcaca gcaagtacag  
 181 // ggccggcggc ttcgcgtccc ggtacgcgcg acgggcacac agcaggacag ccttgctcgg  
 // ccgcccgcgc aagcgcgagg ccatgcgcgc tgcccgtgtg tcgtccgtgc ggaacaggcc  
 241 ctcgattatc ataaacaatc ctgcaggcat gcaagctgga tcatacaag tttgtacaaa  
 gagctaatag tatttgtag gacgtccgta cgttcgacct agtaggttc aaacatgttt  
 Int V





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## pDEST19 6668 bp (rotated to position 1000)

Location (Base Nos.)	Gene Encoded
515..391	attR1
765..1424	CmR
1544..1628	inactivated ccdA
1766..2071	ccdB
2112..2236	attR2
2852..2895	lacZ
3344..4319	ampR
4460..5114	ori
5608..52	genR

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1 AGTGGTTCGC ATCCTCGGTT TTCTGGAAGG CGAGCATCGT TTGTTGCCCC AGGACTCTAG
61 CTATAGTTCT AGTGGTTGGC TACGTATATC AAATACTTGT AGGTGACGCC GTCATCTTTC
121 CATTGTAACG TAAATGGCAA CTTGTAGATG AACGCGCTGT CAAAAAACCG GCCAGTTTCT
181 TCCACAAACT CGCGCACGGC TGTCTCGTAA ACTTTTGCGT CGCAACAATC GCGATGACCT
241 CGTGGTATGG AAATTTTTTC TAAAAAAGTG TCGTTCATGT CCGCGGCGGG CCGGTTTCGG
301 CTCGGTACG CGCGACGGG ACACAGCAGG ACAGCCTTGT CCGGCTCGAT TATCATAAAC
361 AATCCTGCAG GCATGCAAGC TCGGATCATC ACAAGTTTGT ACAAAAAAGC TGAACGAGAA
421 ACGTAAAATG ATATAAATAT CAATATATTA AATTAGATTT TGCATAAAAA ACAGACTACA
481 TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC CGCTAAGTTG GCAGCATCAC
541 CCGACGCACT TTGCGCCGAA TAAATACCTG TGACGGAAGA TCACTTCGCA GAATAAATAA
601 ATCCTGGTGT CCCTGTTGAT ACCGGGAAGC CCTGGGCCAA CTTTGTGCGA AAATGAGACG
661 TTGATCGGCA CGTAAGAGGT TCCAACTTTC ACCATAATGA AATAAGATCA CTACCGGGCG
721 TATTTTTTGA GTTATCGAGA TTTTCAGGAG CTAAGGAAGC TAAAATGGAG AAAAAATCA
781 CTGGATATAC CACCGTTGAT ATATCCCAAT GGCATCGTAA AGAACATTTT GAGGCATTTT
841 AGTCAGTTGC TCAATGTACC TATAACCAGA CCGTTCAGCT GGATATTACG GCCTTTTTAA
901 AGACCGTAAA GAAAAATAAG CACAAGTTT ATCCGGCCTT TATTCACATT CTGCCCCGCC
961 TGATGAATGC TCATCCGGA TCCCGTATGG CAATGAAAGA CCGTGAGCTG GTGATATGGG
1021 ATAGTGTTCA CCCTTGTTAC ACCGTTTTC ATGAGCAAAC TGAAACGTTT TCATCGCTCT
1081 GGAGTGAATA CCACGACGAT TTCCGGCAGT TTCTACACAT ATATTCGCAA GATGTGGCGT
1141 GTTACGGTGA AAACCTGGCC TATTTCCCTA AAGGGTTTAT TGAGAATATG TTTTTCGTCT
1201 CAGCCAATCC CTGGGTGAGT TTCACCAGTT TTGATTTAAA CGTGGCCAAT ATGGACAAC
1261 TCTTCGCCCC CGTTTTACAC ATGGGCAAAT ATTATACGCA AGGCGACAAG GTGCTGATGC
1321 CGCTGGCGAT TCAGGTTTCA CATGCCGTCT GTGATGGCTT CCATGTCGGC AGAATGCTTA
1381 ATGAATTACA ACAGTACTGC GATGAGTGGC AGGGCGGGGC GTAAACGCGT GGATCCGGCT
1441 TACTAAAAGC CAGATAACAG TATGCGTATT TGCGCGCTGA TTTTGTGCGT ATAAGAATAT
1501 ATACTGATAT GTATACCCGA AGTATGTCAA AAAGAGGTGT GCTATGAAGC AGCGTATTAC
1561 AGTGACAGTT GACAGCGACA GCTATCAGTT GCTCAAGGCA TATATGATGT CAATATCTCC
1621 GGTCTGGTAA GCACAACCAT GCAGAATGAA GCCCGTCGTC TCGTGCCGA ACGCTGGAAA
1681 GCGGAAAATC AGGAAGGGAT GGCTGAGGTC GCCCGGTTTA TTGAAATGAA CGGCTCTTTT
1741 GCTGACGAGA ACAGGGACTG GTGAAATGCA GTTTAAGGTT TACACCTATA AAAGAGAGAG
1801 CCGTTATCGT CTGTTTGTGG ATGTACAGAG TGATATTATT GACACGCCCC GGCGACGGAT
1861 GGTGATCCCC CTGGCCAGTG CACGTCTGCT GTCAGATAAA GTCTCCCGTG AACTTTACCC
1921 GGTGGTGCAT ATCGGGGATG AAAGCTGGCG CATGATGACC ACCGATATGG CCAGTGTGCC
1981 GGTCTCCGTT ATCGGGGAAG AAGTGGCTGA TCTCAGCCAC CGCGAAAATG ACATCAAAAA
2041 CGCCATTAACT CTGATGTTCT GGGGAATATA AATGTCAGGC TCCCTTATAC ACAGCCAGTC
2101 TGCAGGTCGA CCATAGTGAC TGGATATGTT GTGTTTTACA GTATTATGTA GTCTGTTTTT
2161 TATGCAAAAT CTAATTTAAT ATATTGATAT TTATATCATT TTACGTTTCT CGTTCAGCTT
2221 TCTTGTAACA AGTGGTGATC GAGAAGTACT AGAGGATCAT AATCAGCCAT ACCACATTTG
2281 TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA
2341 TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA
2401 ATAGCATCAC AAATTTTACA AATAAAGCAT TTTTTCCTACT GCATTCTAGT TGTGGTTTGT
2461 CCAAATCAT CAATGTATCT TATCATGTCT GGATCTGATC ACTGCTTGAG CCTAGGAGAT
2521 CCGAACCAGA TAAGTGAAAT CTAGTTCCAA ACTATTTTGT CATTTTTAAT TTTCGTATTA
2581 GCTTACGACG CTACACCCAG TTCCCATCTA TTTTGTCACT CTTCCTTAAA TAATCCTTAA-

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FIGURE 39B

105/240

2641 AAACCTCCATT TCCACCCCTC CCAGTTCCCA ACTATTTTGT CCGCCACAG CGGGGCATTT  
2701 TTCCTCCTGT TATGTTTTTA ATCAAACATC CTGCCAACTC CATGTGACAA ACCGTCATCT  
2761 TCGGCTACTT TTTCTCTGTC ACAGAATGAA AATTTTCTG TCATCTCTTC GTTATTAATG  
2821 TTTGTAATTG ACTGAATATC AACGCTTATT TGCAGCCTGA ATGGCGAATG GACGCGCCCT  
2881 GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG  
2941 CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC CTTTCTCGCC ACGTTCGCGG  
3001 GCTTTCCTCG TCAAGCTCTA AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC  
3061 GGCACCTCGA CCCCCAAAAA CTTGATTAGG GTGATGGTTC ACGTAGTGGG CCATCGCCCT  
3121 GATAGACGGT TTTTCGCCCT TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT  
3181 TCCAACTGG AACAACTC AACCTATCT CGGTCTATTC TTTTGATTTA TAAGGGATTT  
3241 TGCCGATTTT GGCCTATTGG TTAATAAATG AGCTGATTTA AAAAAATTT AACGCGAATT  
3301 TTAACAAAAT ATTAACGTTT ACAATTTTCA GTGGCACTTT TCGGGGAAAT GTGCGCGGAA  
3361 CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC  
3421 CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAG CATTTCCGTG  
3481 TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC  
3541 TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTAC ATCGAACTGG  
3601 ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA  
3661 GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC  
3721 AACTCGGTCG CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG  
3781 AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA  
3841 GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG  
3901 CTTTTTTGCA CAACATGGGG GATCATGTAA CTCGCCCTGA TCGTTGGGAA CCGGAGCTGA  
3961 ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC TGAGCAATG GCAACAACGT  
4021 TGCGCAAACT ATTAACCTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT  
4081 GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT  
4141 TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CCGTATCATT GCAGCACTGG  
4201 GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA  
4261 TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAC  
4321 TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA  
4381 AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT  
4441 TTTCGTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT  
4501 TTTTCTGCG CGTAATCTGC TGCTTGCAA CAAAAAACC ACCGCTACCA GCGGTGGTTT  
4561 GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC  
4621 AGATACCAA TACTGTCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG  
4681 TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG  
4741 ATAAGTCGTG TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT  
4801 CGGGCTGAAC GGGGGGTTCG TGCACACAGC CCAGCTTGA GCGAACGACC TACACCGAAC  
4861 TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCGAAGGG AGAAAGGCGG  
4921 ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG  
4981 GAAACGCCTG GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT GAGCGTCGAT  
5041 TTTTGTGATG CTCGTACGGG GGGCGGAGCC TATGGAAAAA CGCCAGCAAC GCGGCCTTTT  
5101 TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCTGCG TTATCCCCTG  
5161 ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA  
5221 CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCTGATG CCGTATTTTC  
5281 TCCTTACGCA TCTGTGCGGT ATTTACACAC GCAGACCAGC CGCGTAACCT GGCAAAATCG  
5341 GTTACGGTTG AGTAATAAAT GGATGCCCTG CGTAAGCGGG TGTGGGCGGA CAATAAAGTC  
5401 TTAAACTGAA CAAAATAGAT CTAAACTATG ACAATAAAGT CTTAAACTAG ACAGAATAGT  
5461 TGTAACCTGA AATCAGTCCA GTTATGCTGT GAAAAAGCAT ACTGGACTTT TGTTATGGCT  
5521 AAAGCAAACCT CTTCAATTTTC TGAAGTGCAA ATTGCCCGTC GTATTAAAGA GGGGCGTGGC  
5581 CAAGGGCATG GTAAAGACTA TATTGCGGGC GTTGTGACAA TTTACCGAAC AACTCCGCGG  
5641 CCGGGAAGCC GATCTCGGCT TGAACGAATT GTTAGGTGGC GGTACTTGGG TCGATATCAA  
5701 AGTGCATCAC TTCTTCCCGT ATGCCCAACT TTGTATAGAG AGCCACTGCG GGATCGTCAC  
5761 CGTAATCTGC TTGCACGTAG ATCACATAAG CACCAAGCGC GTTGGCCTCA TGCTTGAGGA  
5821 GATTGATGAG CGCGGTGGCA ATGCCCTGCC TCCGGTGCTC GCCGGAGACT GCGAGATCAT  
5881 AGATATAGAT CTCACTACGC GGCTGCTCAA ACCTGGGCAG AACGTAAGCC GCGAGAGCGC  
5941 CAACAACCGC TTCTTGGTGC AAGGCAGCAA GCGCGATGAA TGTCTTACTA CCGAGCAAGT  
6001 TCCCGAGGTA ATCGGAGTCC GGCTGATGTT GGGAGTAGGT GGCTACGTCT CCGAACTCAC  
6061 GACCGAAAAG ATCAAGAGCA GCCCGCATGG ATTTGACTTG GTCAGGGCCG AGCCTACATG-

Figure 39C

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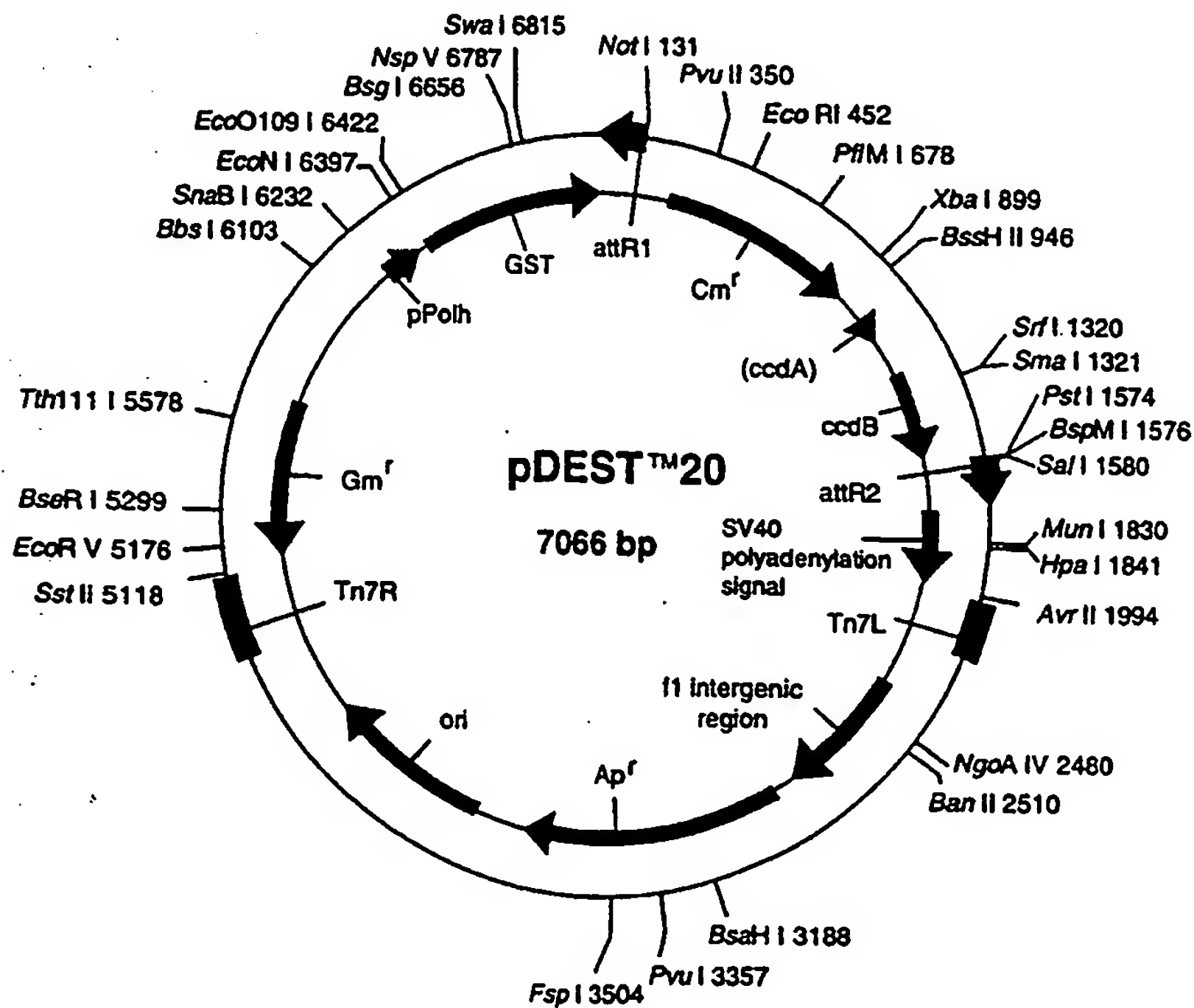
6121 TCGGAATGAT GCCCATACTT GAGCCACCTA ACTTTGTTTT AGGGCGACTG CCCTGCTGCG  
6181 TAACATCGTT GCTGCTGCGT AACATCGTTG CTGCTCCATA ACATCAAACA TCGACCCACG  
6241 GCGTAACGCG CTTGCTGCTT GGATGCCCCG GGCATAGACT GTACAAAAAA ACAGTCATAA  
6301 CAAGCCATGA AAACCGCCAC TGCGCCGTTA CCACCGCTGC GTTCGGTCAA GGTTCTGGAC  
6361 CAGTTGCGTG AGCGCATACG CTACTTGCAT TACAGTTTAC GAACCGAACA GGCTTATGTC  
6421 AACTGGGTTC GTGCCTTCAT CCGTTTCCAC GGTGTGCGTC ACCCGGCAAC CTTGGGCAGC  
6481 AGCGAAGTCG AGGCATTCT GTCTGGCTG GCGAACGAGC GCAAGGTTTC GGTCTCCACG  
6541 CATCGTCAGG CATTGGCGGC CTTGCTGTTT TTCTACGGCA AGGTGCTGTG CACGGATCTG  
6601 CCCTGGCTTC AGGAGATCGG AAGACCTCGG CCGTCGCGGC GCTTGCCGGT GGTGCTGACC  
6661 CCGGATGA

FIGURE 39A

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Figure 40A: pDEST20 Glutathione-S-transferase Fusion with Polyhedron Promoter for Baculovirus Expression

430 ggc tac gta tac tcc gga ata tta ata gat cat gga gat aat taa aat gat  
 cec atg cat atg agg cct tat aat tat cta gta cct cta tta att tta cta  
 481 // aac cat ctc gca aat aaa taa gta ttt tac tgt ttt cgt aac agt ttt gta  
 // ttc gta gag cgt tta ttt att cat aaa atg aca aaa gca ttg tca aaa cat  
 532 // ata aaa aaa cct ata aat att ceg gat tat tca tac cgt ccc acc atc ggg  
 tat ttt ttt gga tat tta taa ggc cta ata agt atg gca ggg tgg tag ccc  
 Start Transl. mRNA → A P T - - - - GST- -  
 583 cgc gga tcc atg gcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg  
 gcg cct agg tac cgg gga tat gat cca ata acc ttt taa ttc ccg gaa cac  
 1246 // S D L V P R H N Q T S L Y K K A  
 tcc gat ctg gtt ccg cgt cat aat caa aca agt ttg tac aaa aaa gct gaa  
 agc cta gac caa ggc gca gta tta gtt tgt tca aac atg ttt ttt cga ctt  
 1297 cga gaa acg taa aat gat ata aat atc aat ata tta aat tag at  
 gct ctt tgc att tta cta tat tta tag tta tat aat tta atc ta



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## pDEST20 7066 bp (rotated to position 5800)

Location (Base Nos.)			Gene Encoded			
		592..1263			GST	
		1397..1273			attR1	
		1506..2165			CmR	
		2285..2369			inactivated ccdA	
		2507..2812			ccdB	
		2853..2977			attR2	
		4214..5064			ampR	
		5263..5843			ori	
1	CCACTGCGCC	GTTACCACCG	CTGCGTTCGG	TCAAGGTTCT	GGACCAGTTG	CGTGAGCGCA
61	TACGCTACTT	GCATTACAGT	TTACGAACCG	AACAGGCTTA	TGTCAACTGG	GTTTCGTGCCT
121	TCATCCGTTT	CCACGGTGTG	CGTCACCCGG	CAACCTTGGG	CAGCAGCGAA	GTCGAGGCAT
181	TTCTGTCCTG	GCTGGCGAAC	GAGCGCAAGG	TTTCGGTCTC	CACGCATCGT	CAGGCATTGG
241	CGGCCTTGCT	GTTCTTCTAC	GGCAAGGTGC	TGTGCACGGA	TCTGCCCTGG	CTTCAGGAGA
301	TCGGAAGACC	TCGGCCGTCG	CGGCGCTTGC	CGGTGGTGCT	GACCCCGGAT	GAAGTGGTTC
361	GCATCCTCGG	TTTTCTGGAA	GGCGAGCATC	GTTTGTTCGC	CCAGGACTCT	AGCTATAGTT
421	CTAGTGGTTG	GCTACGTATA	CTCCGGAATA	TTAATAGATC	ATGGAGATAA	TTAAAATGAT
481	AACCATCTCG	CAAATAAATA	AGTATTTTAC	TGTTTTCGTA	ACAGTTTTGT	AATAAAAAAA
541	CCTATAAATA	TTCCGGATTA	TTCATACCGT	CCCACCATCG	GGCGCGGATC	CATGGCCCCCT
601	ATACTAGGTT	ATTGGAAAAT	TAAGGGCCTT	GTGCAACCCA	CTCGACTTCT	TTTGGAATAT
661	CTTGAAGAAA	AATATGAAGA	GCATTTGTAT	GAGCGCGATG	AAGGTGATAA	ATGGCGAAAC
721	AAAAAGTTTG	AATTGGGTTT	GGAGTTTCCC	AATCTTCCTT	ATTATATTGA	TGGTGATGTT
781	AAATTAACAC	AGTCTATGGC	CATCATACTG	TATATAGCTG	ACAAGCACAA	CATGTTGGGT
841	GGTTGTCCAA	AAGAGCGTGC	AGAGATTTCA	ATGCTTGAAG	GAGCGGTTTT	GGATATTAGA
901	TACGGTGTTT	CGAGAATTGC	ATATAGTAAA	GACTTTGAAA	CTCTCAAAGT	TGATTTTCTT
961	AGCAAAGCTAC	CTGAAATGCT	GAAAATGTTC	GAAGATCGTT	TATGTCATAA	AACATATTTA
1021	AATGGTGATC	ATGTAACCCA	TCCTGACTTC	ATGTTGTATG	ACGCTCTTGA	TGTTGTTTTA
1081	TACATGGACC	CAATGTGCCT	GGATGCGTTC	CCAAAATTAG	TTTGTTTTAA	AAAACGTATT
1141	GAAGCTATCC	CACAAATTGA	TAAGTACTTG	AAATCCAGCA	AGTATATAGC	ATGGCCTTTG
1201	CAGGGCTGGC	AAGCCACGTT	TGGTGGTGGC	GACCATCCTC	CAAAATCGGA	TCTGGTTCCG
1261	CGTCATAATC	AAACAAGTTT	GTACAAAAAA	GCTGAACGAG	AAACGTAAAA	TGATATAAAT
1321	ATCAATATAT	TAAATTAGAT	TTTGCATAAA	AAACAGACTA	CATAATACTG	TAAAACACAA
1381	CATATCCAGT	CACTATGGCG	GCCGCATTAG	GCACCCAGG	CTTTACACTT	TATGCTTCCG
1441	GCTCGTATGT	TGTGTGGATT	TTGAGTTAGG	ATCCGGCGAG	ATTTTCAGGA	GCTAAGGAAG
1501	CTAAAATGGA	GAAAAAATC	ACTGGATATA	CCACCGTTGA	TATATCCCAA	TGGCATCGTA
1561	AAGAACATTT	TGAGGCATTT	CAGTCAGTTG	CTCAATGTAC	CTATAACCAG	ACCGTTCAGC
1621	TGGATATTAC	GGCCTTTTTA	AAGACCGTAA	AGAAAAATAA	GCACAAGTTT	TATCCGGCCT
1681	TTATTCACAT	TCTTGCCCGC	CTGATGAATG	CTCATCCGGA	ATTCCGTATG	GCAATGAAAG
1741	ACGGTGAGCT	GGTGATATGG	GATAGTGTTT	ACCCTTGTTA	CACCGTTTTT	CATGAGCAAA
1801	CTGAAACGTT	TTCATCGCTC	TGGAGTGAAT	ACCACGACGA	TTTCCGGCAG	TTTCTACACA
1861	TATATTGCGA	AGATGTGGCG	TGTTACGGTG	AAAACCTGGC	CTATTTCCCT	AAAGGGTTTA
1921	TTGAGAATAT	GTTTTTCGTC	TCAGCCAATC	CCTGGGTGAG	TTTCACCAGT	TTTGATTTAA
1981	ACGTGGCCAA	TATGGACAAC	TTCTTCGCCC	CCGTTTTTCAC	CATGGGCAAA	TATTATACGC
2041	AAGGCGACAA	GGTGCTGATG	CCGCTGGCGA	TTCAGGTTCA	TCATGCCGTC	TGTGATGGCT
2101	TCCATGTCGG	CAGAATGCTT	AATGAATTAC	AACAGTACTG	CGATGAGTGG	CAGGGCGGGG
2161	CGTAATCTAG	AGGATCCGGC	TTACTAAAAG	CCAGATAACA	GTATGCGTAT	TTGCGCGCTG
2221	ATTTTTGCGG	TATAAGAATA	TATACTGATA	TGTATACCCG	AAGTATGTCA	AAAAGAGGTG
2281	TGCTATGAAG	CAGCGTATTA	CAGTGACAGT	TGACAGCGAC	AGCTATCAGT	TGCTCAAGGC
2341	ATATATGATG	TCAATATCTC	CGGTCTGGTA	AGCACAACCA	TGCAGAATGA	AGCCCGTCGT
2401	CTGCGTGCCG	AACGCTGGAA	AGCGGAAAAT	CAGGAAGGGA	TGGCTGAGGT	CGCCCGGTTT
2461	ATTGAAATGA	ACGGCTCTTT	TGCTGACGAG	AACAGGGACT	GGTGAAATGC	AGTTTAAGGT
2521	TTACACCTAT	AAAAGAGAGA	GCCGTTATCG	TCTGTTTGTG	GATGTACAGA	GTGATATTAT
2581	TGACACGCCC	GGGCGACGGA	TGGTGATCCC	CCTGGCCAGT	GCACGTCTGC	TGTCAGATAA
2641	AGTCTCCCGT	GAACTTTACC	CGGTGGTGCA	TATCGGGGAT	GAAAGCTGGC	GCATGATGAC

Figure 40B



2701 CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG ATCTCAGCCA  
2761 CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT TGGGGAATAT AAATGTCAGG  
2821 CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA CTGGATATGT TGTGTTTTAC  
2881 AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA TTTATATCAT  
2941 TTTACGTTTC TCGTTCAGCT TTCTTGATCA AAGTGGTTTG ATAGCTTGTC GAGAAGTACT  
3001 AGAGGATCAT AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA AAAAACCTCC  
3061 CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTGTTGT AACTTGTTTA  
3121 TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTTACA AATAAAGCAT  
3181 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAATCAT CAATGTATCT TATCATGTCT  
3241 GGATCTGATC ACTGCTTGAG CCTAGGAGAT CCGAACCAGA TAAGTGAAAT CTAGTTCCAA  
3301 ACTATTTTGT CATTTTTAAT TTTCGTATTA GCTTACGACG CTACACCCAG TTCCCATCTA  
3361 TTTTGTCACT CTTCCCTAAA TAATCCTTAA AAATCCATT TCCACCCCTC CCAGTTCCCA  
3421 ACTATTTTGT CCGCCACAG CGGGGCATTT TTCTTCCTGT TATGTTTTTA ATCAAACATC  
3481 CTGCCAACTC CATGTGACAA ACCGTCTATCT TCGGCTACTT TTTCTCTGTC ACAGAATGAA  
3541 AATTTTTCTG TCATCTCTTC GTTATTAATG TTTGTAATTG ACTGAATATC AACGCTTATT  
3601 TGCAGCCTGA ATGGCGAATG GACGCGCCCT GTAGCGGCGC ATTAAGCGCG GCGGGTGTGG  
3661 TGGTTACGCG CAGCGTGACC GCTACACTTG CCAGCGCCCT AGCGCCCGCT CTTTTCGCTT  
3721 TCTTCCCTTC CTTTCTCGCC ACGTTCGCCG GCTTTCCTCG TCAAGCTCTA AATCGGGGGC  
3781 TCCCTTTAGG GTTCCGATTT AGTGCTTTAC GGCACCTCGA CCCCCAAAAA CTTGATTAGG  
3841 GTGATGGTTC ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT TTGACGTTGG  
3901 AGTCCACGTT CTTTAATAGT GGACTCTTGT TCCAACTGG AACAACACTC AACCTATCT  
3961 CGGTCTATTC TTTTGATTTA TAAGGGATTT TGCCGATTTT GGCCTATTGG TTAAAAAATG  
4021 AGCTGATTTA ACAAAAATTT AACGCGAATT TTAACAAAAT ATTAACGTTT ACAATTTTCA  
4081 GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT  
4141 CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA  
4201 GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTT GCGGCATTTT  
4261 GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT  
4321 TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT  
4381 TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG  
4441 TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCG CCGCATACAC TATTCTCAGA  
4501 ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA  
4561 GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTAATTCTGA  
4621 CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA CAACATGGGG GATCATGTAA  
4681 CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA  
4741 CCACGATGCC TGTAAGCAATG GCAACAACGT TGCGCAAAC ATTAAGTGGC GAACTACTTA  
4801 CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC  
4861 TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC  
4921 GTGGGTCTCG CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG  
4981 TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA  
5041 TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT  
5101 AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA  
5161 ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG  
5221 AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA  
5281 CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT  
5341 TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCCTT CTAGTGTAGC  
5401 CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCACC TACATACCTC GCTCTGCTAA  
5461 TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA  
5521 GACGATAGTT ACCGGATAAG GCGCAGCGGT CCGGCTGAAC GGGGGGTTTC TGCACACAGC  
5581 CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA  
5641 GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA  
5701 CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTGC  
5761 GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC  
5821 TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTTCT GGCCTTTTGC TGGCCTTTTG  
5881 CTCACATGTT CTTTCTGCG TTATCCCTG ATTCTGTGGA TAACCGTATT ACCGCTTTTG  
5941 AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG  
6001 AAGCGGAAGA GCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACCC  
6061 GCAGACCAGC CGCGTAACCT GGCAAAATCG GTTACGGTTG AGTAATAAAT GGATGCCCTG  
6121 CGTAAGCGGG TGTGGGCGGA CAATAAAGTC TTAAACTGAA CAAAATAGAT CTAAACTATG-



6181 ACAATAAAGT CTTAAACTAG ACAGAATAGT TGTAAGTGA AATCAGTCCA GTTATGCTGT  
6241 GAAAAAGCAT ACTGGACTTT TGTTATGGCT AAAGCAAACCT CTTCAATTTTC TGAAGTGCAA  
6301 ATTGCCCGTC GTATTAAAGA GGGGCGTGGC CAAGGGCATG GTAAAGACTA TATTCGCGGC  
6361 GTTGTGACAA TTTACCGAAC AACTCCGCGG CCGGGAAGCC GATCTCGGCT TGAACGAATT  
6421 GTTAGGTGGC GGTACTTGGG TCGATATCAA AGTGCATCAC TTCTTCCCGT ATGCCCAACT  
6481 TTGTATAGAG AGCCACTGCG GGATCGTCAC CGTAATCTGC TTGCACGTAG ATCACATAAG  
6541 CACCAAGCGC GTTGGCCTCA TGCTTGAGGA GATTGATGAG CGCGGTGGCA ATGCCCTGCC  
6601 TCCGGTGCTC GCCGGAGACT GCGAGATCAT AGATATAGAT CTCACTACGC GGCTGCTCAA  
6661 ACCTGGGCAG AACGTAAGCC GCGAGAGCGC CAACAACCGC TTCTTGGTCG AAGGCAGCAA  
6721 GCGCGATGAA TGTCTTACTA CGGAGCAAGT TCCCGAGGTA ATCGGAGTCC GGCTGATGTT  
6781 GGGAGTAGGT GGCTACGTCT CCGAACTCAC GACCGAAAAG ATCAAGAGCA GCGCGCATGG  
6841 ATTTGACTTG GTCAGGGCCG AGCCTACATG TCGGAATGAT GCCCATACTT GAGCCACCTA  
6901 ACTTTGTTTT AGGGCGACTG CCCTGCTGCG TAACATCGTT GCTGCTGCGT AACATCGTTG  
6961 CTGCTCCATA ACATCAAACA TCGACCCACG GCGTAACGCG CTTGCTGCTT GGATGCCCGA  
7021 GGCATAGACT GTACAAAAAA ACAGTCATAA CAAGCCATGA AAACCG

FIGURE 40D

Figure 41A:

pDEST21

2-Hybrid Vector with  
DNA-Binding Domain

**ADH Promoter**

700 ~~ttg gcg ctt tgc tat caa gta taa ata gac ctg caa tta tta atc ttt tgt~~  
~~aac ggc gaa acg ata gtt cat att tat ctg gac gtt aat aat tag aaa aca~~

751 ~~ttc ctc gtc att gtt ctc gtt ccc ttt ctt cct tgt ttc ttc ttc tgc aca~~  
~~aag gag cag taa caa gag caa ggg aaa gaa gga aca aag aag aag acg tgt~~

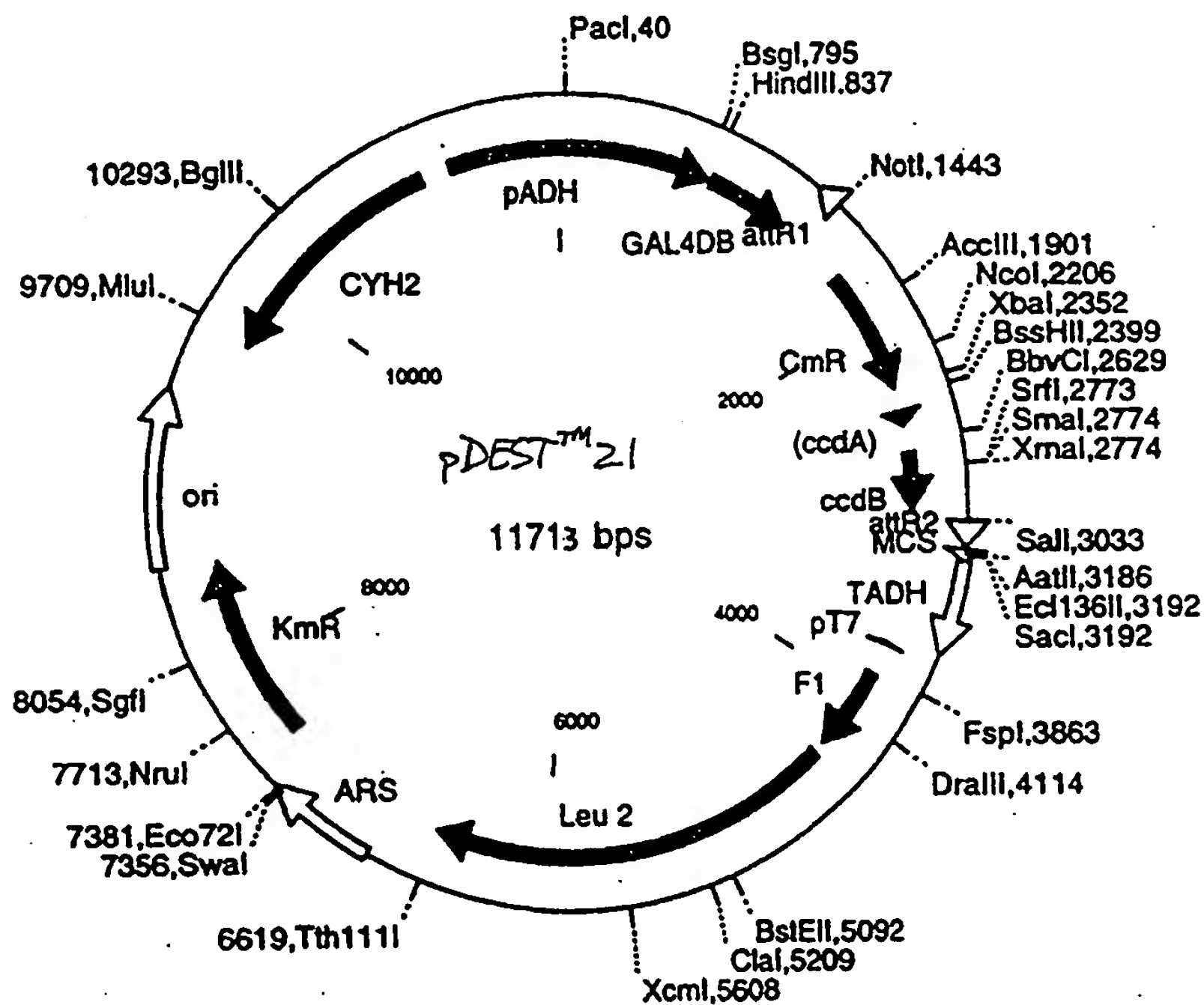
802 ~~ata ttt caa gct ata cca agc ata caa tca act~~ cca agc ttg aag caa gcc  
~~tat aaa gtt cga tat ggt tca tat gtt agt tga~~ ggt tca aac ttc gtt cgg

853 ~~Start Transl~~ **M** **K** **L** **L** **S** **S** **-** **-** **Gal4-DB**  
~~tcc tga aag atg aag cta ctg tct tct atc gaa caa gca tgc gat att tgc~~  
~~agg act ttc tac ttc gat gac aga aga tag ctt gtt cgt acg cta taa acg~~

1261 gaa gag agt agt aac aaa ggt caa aga cag ttg act gta tgc tgc agg tgc  
ctt ctc tca tca ttg ttt cca gtt tct gtc aac tga cat agc agc tcc agc

1312 **N** **Q** **T** **S** **L** **Y** **K** **K** **A** **act R1**  
~~aat caa aca agt ttg tac aaa aaa gct gaa cga gaa acg taa aat gat ata~~  
~~cta gtt tgt tca aac atg ttt ttt cga ctt gct ctt tgc att tta cta tat~~

Int V



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## pDEST21 11713 bp (rotated to position 11000)

Location (Base Nos.)	Gene Encoded
857..1322	GAL4DB
1456..1332	attR1
1706..2365	CmR
2485..2569	inactivated ccdA
2707..3012	ccdB
3053..3177	attR2
3716..3735	pT7 (T7 promoter)
3899..4354	f1 (f1 intergenic region)
4414..6642	Leu2
7541..8515	kanR
9668..10958	CYH2
11118..848	pADH (ADH promoter)

```

1 TTTATTATGT TACAATATGG AAGGGAACCT TACACTTCTC CTATGCACAT ATATTAATTA
61 AAGTCCAATG CTAGTAGAGA AGGGGGGTAA CACCCCTCCG CGCTCTTTTC CGATTTTTTT
121 CTAAACCGTG GAATATTTTCG GATATCCTTT TGTTGTTTCC GGGTGTACAA TATGGACTTC
181 CTCTTTTCTG GCAACCAAAC CCATACATCG GGATTCCTAT AATACCTTCG TTGGTCTCCC
241 TAACATGTAG GTGGCGGAGG GGAGATATAC AATAGAACAG ATACCAGACA AGACATAATG
301 GGCTAAACAA GACTACACCA ATTACACTGC CTCATTGATG GTGGTACATA ACGAACTAAT
361 ACTGTAGCCC TAGACTTGAT AGCCATCATC ATATCGAAGT TTCACTACCC TTTTTCATT
421 TGCCATCTAT TGAAGTAATA ATAGGCGCAT GCAACTTCTT TTCTTTTTTT TTCTTTTCTC
481 TCTCCCCCGT TGTTGTCTCA CCATATCCGC AATGACAAAA AAAATGATGG AAGACACTAA
541 AGGAAAAAAT TAACGACAAA GACAGCACCA ACAGATGTCG TTGTTCCAGA GCTGATGAGG
601 GGTATCTTCG AACACACGAA ACTTTTTCTT TCCTTCATTC ACGCACACTA CTCTCTAATG
661 AGCAACGGTA TACGGCCTTC CTTCCAGTTA CTTGAATTTG AAATAAAAAA AGTTTGCCGC
721 TTTGCTATCA AGTATAAATA GACCTGCAAT TATTAATCTT TTGTTTCTC GTCATGTTC
781 TCGTTCCTT TCTTCCTTGT TTCTTTTTCT GCACAATATT TCAAGCTATA CCAAGCATAC
841 AATCAACTCC AAGCTTGAAG CAAGCCTCCT GAAAGATGAA GCTACTGTCT TCTATCGAAC
901 AAGCATGCGA TATTTGCCGA CTTAAAAAGC TCAAGTGCTC CAAAGAAAAA CCGAAGTGCG
961 CCAAGTGTCT GAAGAACAAC TGGGAGTGTC GCTACTCTCC CAAAACCAAA AGGTCTCCGC
1021 TGA TAGGGC ACATCTGACA GAAGTGGAAT CAAGGCTAGA AAGACTGGAA CAGCTATTTT
1081 TACTGATTTT TCCTCGAGAA GACCTTGACA TGATTTTGAA AATGGATTCT TTACAGGATA
1141 TAAAAGCATT GTTAACAGGA TTATTTGTAC AAGATAATGT GAATAAAGAT GCCGTCACAG
1201 ATAGATTGGC TTCAGTGGAG ACTGATATGC CTCTAACATT GAGACAGCAT AGAATAAGTG
1261 CGACATCATC ATCGGAAGAG AGTAGTAACA AAGGTCAAAG ACAGTTGACT GTATCGTCGA
1321 GGTGCAATCA AACAAAGTTG TACAAAAAAG CTGAACGAGA AACGTAAAT GATATAAATA
1381 TCAATATATT AAATTAGATT TTGCATAAAA AACAGACTAC ATAATACTGT AAAACACAAC
1441 ATATCCAGTC ACTATGGCGG CCGCTAAGTT GGCAGCATCA CCGACGCAC TTTGCGCCGA
1501 ATAAATACCT GTGACGGAAG ATCACTTCGC AGAATAAATA AATCCTGGTG TCCCTGTTGA
1561 TACCGGGAAG CCCTGGGCCA ACTTTTGGCG AAAATGAGAC GTTGATCGGC ACGTAAGAGG
1621 TTCCAACCTT CACCATAATG AAATAAGATC ACTACGGGC GTATTTTTTG AGTTATCGAG
1681 ATTTTCAGGA GCTAAGGAAG CTAAAATGGA GAAAAAATC ACTGGATATA CCACCGTTGA
1741 TATATCCCAA TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG CTCAATGTAC
1801 CTATAACCAG ACCGTTTCAGC TGGATATTAC GGCCTTTTTA AAGACCGTAA AGAAAAATAA
1861 GCACAAGTTT TATCCGGCCT TTATTCACAT TCTTGCCCGC CTGATGAATG CTCATCCGGA
1921 ATTCCGTATG GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTT ACCCTTGTTA
1981 CACCGTTTTT CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT ACCACGACGA
2041 TTTCCGGCAG TTTCTACACA TATATTCGCA AGATGTGGCG TGTACGGTG AAAACCTGGC
2101 CTATTTCCCT AAAGGGTTTA TTGAGAATAT GTTTTTCGTC TCAGCCAATC CCTGGGTGAG
2161 TTTCAACAGT TTTGATTTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC CCGTTTTTCAC
2221 CATGGGCAAA TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA TTCAGGTTCA
2281 TCATGCCGTC TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC AACAGTACTG
2341 CGATGAGTGG CAGGGCGGGG CGTAATCTAG AGGATCCGGC TTACTAAAAG CCAGATAACA
2401 GTATGCGTAT TTGCGCGCTG ATTTTTCGGG TATAAGAATA TATACTGATA TGTATACCCG-

```

FIGURE 413

2461 AAGTATGTCA AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC  
2521 AGCTATCAGT TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA  
2581 TGCAGAATGA AGCCCGTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAAT CAGGAAGGGA  
2641 TGGCTGAGGT CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT  
2701 GGTGAAATGC AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG  
2761 GATGTACAGA GTGATATTAT TGACACGCCC GGGCGACGGA TGGTGATCCC CCTGGCCAGT  
2821 GCACGTCTGC TGTCAGATAA AGTCTCCCGT GAACTTTACC CGGTGGTGCA TATCGGGGAT  
2881 GAAAGCTGGC GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA  
2941 GAAGTGGCTG ATCTCAGCCA CCGCGAAAAT GACATCAAAA ACGCCATTAA CCTGATGTTC  
3001 TGGGGAATAT AAATGTCAGG CTCCCTTATA CACAGCCAGT CTGCAGGTCG ACCATAGTGA  
3061 CTGGATATGT TGTGTTTAC AGTATTATGT AGTCTGTTTT TTATGCAAAA TCTAATTTAA  
3121 TATATTGATA TTTATATCAT TTTACGTTTC TCGTTCAGCT TTCTTGTAACA AAGTGGTTTG  
3181 ATGGCCGCTA AGTAAGTAAG ACGTCGAGCT CTAAGTAAGT AACGGCCGCC ACCGCGGTGG  
3241 AGCTTTGGAC TTCTTCGCCA GAGGTTTGGT CAAGTCTCCA ATCAAGGTTG TCGGCTTGTC  
3301 TACCTTGCCA GAAATTTACG AAAAGATGGA AAAGGGTCAA ATCGTTGGTA GATACGTTGT  
3361 TGACACTTCT AAATAAGCGA ATTTCTTATG ATTTATGATT TTTATTATTA AATAAGTTAT  
3421 AAAAAAATA AGTGATATAA AATTTTAAAG TGAATCTTAG GTTTTAAAC GAAAATTCTT  
3481 ATTCTTGAGT AACTCTTTC TGTAGGTCAG GTTGCTTTCT CAGGTATAGC ATGAGGTCCG  
3541 TCTTATTGAC CACACCTCTA CCGGCATGCC GAGCAAATGC CTGCAAATCG CTCCCCATT  
3601 CACCCAATTG TAGATATGCT AACTCCAGCA ATGAGTTGAT GAATCTCGGT GTGTATTTTA  
3661 TGTCTCAGA GGACAATACC TGTGTAAATC GTTCTTCCAC ACGGATCCCA ATTCGCCCTA  
3721 TAGTGAGTCG TATTACAATT CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC  
3781 TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCT TTCGCCAGCT GGCCTAATAG  
3841 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG GCGAATGGAC  
3901 GCGCCCTGTA GCGGCGCATT AAGCGCGGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT  
3961 AACTTGCCA GCGCCCTAGC GCCCGCTCCT TTCGCTTTCT TCCCTTCCTT TCTCGCCACG  
4021 TTCGCCGCTT TTCCCGTCA AGCTCTAAAT CGGGGGCTCC CTTTAGGGTT CCGATTTAGT  
4081 GCTTTACGGC ACCTCGACCC CAAAAAATT GATTAGGGTG ATGGTTCACG TAGTGGGCCA  
4141 TCGCCCTGAT AGACGGTTTT TCGCCCTTTG ACGTTGGAGT CCACGTTCTT TAATAGTGGA  
4201 CTCTTGTTCC AAATGGAAC AACACTCAAC CCTATCTCGG TCTATTCTTT TGATTTATAA  
4261 GGGATTTTGC CGATTTCCGC CTATTGGTTA AAAAATGAGC TGATTTAACA AAAATTTAAC  
4321 GCGAATTTTA ACAAATATT AACGTTTACA ATTTCTGAT GCGGTATTTT CTCCTTACGC  
4381 ATCTGTGCGG TATTTCACAC CGCATATCGA CCGGTCGAGG AGAACTTCTA GTATATCCAC  
4441 ATACCTAATA TTATTGCCTT ATTAAAAATG GAATCGGAAC AATTACATCA AAATCCACAT  
4501 TCTCTTCAA ATCAATTGTC CTGTACTTCC TTGTTTCATG GTGTTCAAAA ACGTTATATT  
4561 TATAGGATAA TTATACTCTA TTTCTCAACA AGTAATTGGT TGTTTGGCCG AGCGGTCTAA  
4621 GGCGCCTGAT TCAAGAAATA TCTTGACCGC AGTTAACTGT GGAATACTC AGGTATCGTA  
4681 AGATGCAAGA GTTCGAATCT CTTAGCAACC ATTATTTTTT TCCTCAACAT AACGAGAACA  
4741 CACAGGGGCG CTATCGCACA GAATCAAATT CGATGACTGG AAATTTTTTG TTAATTTTACG  
4801 AGGTGCGCTG ACGCATATAC CTTTTTCAAC TGAAAAATTG GGAGAAAAAG GAAAGGTGAG  
4861 AGGCCGGAAC CGGCTTTTCA TATAGAATAG AGAAGCGTTC ATGACTAAAT GCTTGCATCA  
4921 CAATACTTGA AGTTGACAAT ATTATTTAAG GACCTATTGT TTTTCCAAT AGGTGGTTAG  
4981 CAATCGTCTT ACTTCTAAC TTTCTTACC TTTTACATTT CAGCAATATA TATATATATT  
5041 TCAAGGATAT ACCATTCTAA TGTCTGCCCC TATGTCGTGC CCTAAGAAGA TCGTCGTTTT  
5101 GCCAGGTGAC CACGTTGGTC AAGAAATCAC AGCCGAAGCC ATTAAGGTTT TTAAGCTAT  
5161 TTCTGATGTT CGTTCCAATG TCAAGTTCGA TTTCGAAAAT CATTTAATTG GTGGTGCTGC  
5221 TATCGATGCT ACAGGTGTCC CACTTCCAGA TGAGGCGCTG GAAGCCTCCA AGAAGGTGA  
5281 TGCCGTTTTG TTAGGTGCTG TGGGTGGTCC TAAATGGGGT ACCGGTAGTG TTAGACCTGA  
5341 ACAAGGTTTA CTAAAAATCC GTAAAGAACT TCAATTGTAC GCCAACTTAA GACCATGTAA  
5401 CTTTGCATCC GACTCTCTTT TAGACTTATC TCCAATCAAG CCACAATTG CTAAAGGTAC  
5461 TGACTTCGTT GTTGTCAGAG AATTAGTGGG AGGTATTTAC TTTGGTAAGA GAAAGGAAGA  
5521 CGATGGTGAT GGTGTCGCTT GGGATAGTGA ACAATACACC GTTCCAGAAG TGCAAAGAAT  
5581 CACAAGAATG GCCGCTTTCA TGGCCCTACA ACATGAGCCA CCATTGCCTA TTTGGTCTT  
5641 GGATAAAGCT AATGTTTTGG CCTCTTCAAG ATTATGGAGA AAAACTGTGG AGGAAACCAT  
5701 CAAGAACGAA TTCCCTACAT TGAAGGTTCA ACATCAATTG ATTGATTCTG CCGCCATGAT  
5761 CCTAGTTAAG AACCCAACCC ACCTAAATGG TATTATAATC ACCAGCAACA TGTTTGGTGA  
5821 TATCATCTCC GATGAAGCCT CCGTTATCCC AGGTTCTTGG GGTGTTGTTG CATCTGCGTC  
5881 CTTGGCCTCT TTGCCAGACA AGAACACCGC ATTTGGTTTG TACGAACCAT GCCACGGTTC-

FIGURE 41C



5941 TGCTCCAGAT TTGCCAAAGA ATAAGGTTGA CCTATCGCC ACTATCTTGT CTGCTGCAAT  
6001 GATGTTGAAA TTGTCATTGA ACTTGCCTGA AGAAGGTAAG GCCATTGAAG ATGCAGTTAA  
6061 AAAGGTTTTG GATGCAGGTA TCAGAACTGG TGATTTAGGT GGTTCACAACA GTACCACCGA  
6121 AGTCGGTGAT GCTGTCGCCG AAGAAGTTAA GAAAATCCTT GCTTAAAAAG ATTCTCTTTT  
6181 TTTATGATAT TTGTACATAA ACTTTATAAA TGAAATTCAT AATAGAAACG ACACGAAATT  
6241 ACAAATGGA ATATGTTTCAT AGGGTAGACG AAACATATA CGCAATCTAC ATACATTTAT  
6301 CAAGAAGGAG AAAAAGGAGG ATAGTAAAGG AATACAGGTA AGCAAATTGA TACTAATGGC  
6361 TCAACGTGAT AAGGAAAAAG AATTGCACCT TAACATTAAT ATTGACAAGG AGGAGGGCAC  
6421 CACACAAAAA GTTAGGTGTA ACAGAAAATC ATGAAACTAC GATTCCTAAT TTGATATTGG  
6481 AGGATTTTCT CTAAAAAAA AAAAATACAA CAAATAAAAA AACTCAATG ACCTGACCAT  
6541 TTGATGGAGT TTAAGTCAAT ACCTTCTTGA ACCATTTCCT ATAATGGTGA AAGTTCCCTC  
6601 AAGAATTTTA CTCTGTCAGA AACGGCCTTA CGACGTAGTC GATATGGTGC ACTCTCAGTA  
6661 CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA CCCGCCAACA CCCGCTGACG  
6721 CGCCCTGACG GGCTTGCTG CTCCCGGCAT CCGCTTACAG ACAAGCTGTG ACCGTCTCCG  
6781 GGAGCTGCAT GTGTCAGAGG TTTTCACCGT CATCACCGAA ACGCGCGAGA CGAAAGGGCC  
6841 TCGTGATACG CCTATTTTGA TAGGTTAATG TCATGATAAT AATGGTTTCT TAGGACGGAT  
6901 CGCTTGCTG TAACTTACAC GCGCCTCGTA TCTTTTAATG ATGGAATAAT TTGGGAATTT  
6961 ACTCTGTGTT TATTTATTTT TATGTTTGT ATTTGGATT TAGAAAGTAA ATAAAGAAGG  
7021 TAGAAGAGTT ACGGAATGAA GAAAAAATA TAAACAAAGG TTTAAAAAT TTCAACAAAA  
7081 AGCGTACTTT ACATATATAT TTATTAGACA AGAAAAGCAG ATTAAATAGA TATACATTCC  
7141 ATTAACGATA AGTAAATGT AAAATCACAG GATTTTCGTG TGTGGTCTTC TACACAGACA  
7201 AGATGAAACA ATTCGGCATT AATACCTGAG AGCAGGAAGA GCAAGATAAA AGGTAGTATT  
7261 TGTTGGCGAT CCCCCTAGAG TCTTTTACAT CTTCCGAAAA CAAAACTAT TTTTCTTTA  
7321 ATTTCTTTTT TACTTTCTA TTTTAAATTT ATATATTTAT ATTAAAAAT TTAAATTATA  
7381 ATTATTTTTA TAGCACGTGA TGAAAAGGAC CCAGGTGGCA CTTTTCGGGG AAATGTGCGC  
7441 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA  
7501 TAACCCTGAT AAATGCTTCA ATAATCTGCA GCTCTGGCCC GTGTCTCAA ATCTCTGATG  
7561 TTACATTGCA CAAGATAAAA ATATATCATC ATGAACAATA AACTGTCTG CTTACATAAA  
7621 CAGTAATACA AGGGGTGTTA TGAGCCATAT TCAACGGGAA ACGTCTTGCT GGAGGCCGCG  
7681 ATTAAATTCC AACATGGATG CTGATTTATA TGGGTATAAA TGGGCTCGCG ATAATGTCGG  
7741 GCAATCAGGT GCGACAATCT TTCGATTGTA TGGGAAGCCC GATGCGCCAG AGTTGTTTCT  
7801 GAAACATGGC AAAGGTAGCG TTGCCAATGA TGTTACAGAT GAGATGGTCA GACTAACTG  
7861 GCTGACGGAA TTTATGCCTC TTCCGACCAT CAAGCATTTT ATCCGTACTC CTGATGATGC  
7921 ATGGTTACTC ACCACTGCGA TCCGCGGGAA AACAGCATT CAGGTATTAG AAGAATATCC  
7981 TGATTCAGGT GAAAATATTG TTGATGCGCT GGCAGTGTT CTGCGCCGGT TGCATTGAT  
8041 TCCTGTTTGT AATTGTCCTT TTAACAGCGA TCGCGTATTT CGTCTCGCTC AGGCGCAATC  
8101 ACGAATGAAT AACGGTTTGG TTGATGCGAG TGATTTTGAT GACGAGCGTA ATGGCTGGCC  
8161 TGTTGAACAA GTCTGGAAG AAATGCATAC GCTTTTGCCA TTCTCACCAG ATTCAGTCGT  
8221 CACTCATGGT GATTTCTCAC TTGATAACCT TATTTTGGAC GAGGGGAAAT TAATAGGTTG  
8281 TATTGATGTT GGACGAGTCG GAATCGCAGA CCGATACCAG GATCTTGCCA TCCTATGGAA  
8341 CTGCCTCGGT GAGTTTCTC CTTCATTACA GAAACGGCTT TTTCAAAAAT ATGGTATTGA  
8401 TAATCCTGAT ATGAATAAAT TGCAGTTTCA TTTGATGCTC GATGAGTTTT TCTAATCAGA  
8461 ATTGGTTAAT TGGTTGTAAC ACTGGCAGAG CATTACGCTG ACTTGACGGG ACGGCGCATG  
8521 ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC  
8581 AAAGGATCTT CTTGAGATCC TTTTCTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAA  
8641 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTCCGAAG  
8701 GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA  
8761 GGCCACCACT TCAAGAACTC TGTAGCACC CGTACATACC TCGCTCTGCT AATCCTGTTA  
8821 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGCTTACC CGTTGGACTC AAGACGATAG  
8881 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG  
8941 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCATTGAGA AAGCGCCACG  
9001 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCCG AACAGGAGAG  
9061 CGCAGGAGG AGCTTCCAGG GGGGAACGCC TGGTATCTTT ATAGTCCTGT CGGGTTTCGC  
9121 CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCCGAG CCTATGGAAA  
9181 AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG  
9241 TTCTTTCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT  
9301 GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA  
9361 GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC CGATTCTTA ATGCAGCTGG-

FIGURE 4D

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9421 CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA ACGCAATTAA TGTGAGTTAC  
9481 CTCACTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC CGGCTCCTAT GTTGTGTGGA  
9541 ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG ACCATGATTA CGCCAAGCTC  
9601 GGAATTAACC CTCACTAAAG GGAACAAAAG CTGGTACCGA TCCCGAGCTT TGCAAATTAA  
9661 AGCCTTCGAG CGTCCCAAAA CCTTCTCAAG CAAGGTTTTT AGTATAATGT TACATGCGTA  
9721 CACGCGTCTG TACAGAAAAA AAAGAAAAAT TTGAAATATA AATAACGTTT TTAATACTAA  
9781 CATAACTATA AAAAAATAAA TAGGGACCTA GACTTCAGGT TGTCTAACTC CTTCTTTTTC  
9841 GGTTAGAGCG GATGTGGGGG GAGGGCGTGA ATGTAAGCGT GACATAACTA ATTACATGAT  
9901 ATCGACAAAG GAAAAGGGGC CTGTTTACTC ACAGGCTTTT TTCAAGTAGG TAATTAAGTC  
9961 GTTCTGTCTT TTTCTCTTCT TCAACCCACC AAAGGCCATC TTGGTACTTT TTTTTTTTTT  
10021 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT  
10081 TTTTTTTTTT TTTTTTTTTT TCATAGAAAT AATACAGAAG TAGATGTTGA ATTAGATTAA  
10141 ACTGAAGATA TATAATTTAT TGGAAAATAC ATAGAGCTTT TTGTTGATGC GCTTAAGCGA  
10201 TCAATTCAAC AACACCACCA GCAGCTCTGA TTTTCTCTTC AGCCAACTTG GAGACGAATC  
10261 TAGCTTTGAC GATAACTGGA ACATTTGGAA TTCTACCCTT ACCCAAGATC TTACCGTAAC  
10321 CGGCTGCCAA AGTGTCAATA ACTGGAGCAG TTTCTTAGA AGCAGATTTT AAGTATTGGT  
10381 CTCTCTTGTC TTCTGGGATC AATGTCCACA ATTTGTCCAA GTTCAAGACT GGCTTCCAGA  
10441 AATGAGCTTG TTGCTTGTGG AAGTATCTCA TACCAACCTT ACCGAAATAA CCTGGATGGT  
10501 ATTTATCCAT GTTAATTCTG TGGTGATGTT GACCACCGGC CATACCTCTA CCACCGGGGT  
10561 GCTTTCTGTG CTTACCGATA CGACCTTTAC CGGCTGAGAC GTGACCTCTG TGCTTTCTAG  
10621 TCTTAGTGAA TCTGGAAGGC ATTCTTGATT AGTTGGATGA TTGTTCTGGG ATTTAATGCA  
10681 AAAATCACTT AAGAAGGAAA ATCAACGGAG AAAGCAAACG CCATCTTAAA TATACGGGAT  
10741 ACAGATGAAA GGGTTTGAAC CTATCTGGAA AATAGCATT AACAAGCGAA AAAGTGGAG  
10801 GAAAATTGTT TGCCTCTCTG CGGGCTATTC ACGCGCCAGA GGAAAATAGG AAAAATAACA  
10861 GGGCATTAGA AAAATAATTT TGATTTTGGT AATGTGTGGG TCCTGGTGTA CAGATGTTAC  
10921 ATTGGTTACA GTACTCTTGT TTTTGCTGTG TTTTTCGATG AATCTCCAAA ATGGTTGTTA  
10981 GCACATGGAA GAGTCACCGA TGCTAAGTTA TCTCTATGTA AGCTACGTGG CGTGACTTTT  
11041 GATGAAGCCG CACAAGAGAT ACAGGATTGG CAACTGCAAA TAGAATCTGG GGATCCCCC  
11101 TCGAGATCCG GGATCGAAGA AATGATGGTA AATGAAATAG GAAATCAAGG AGCATGAAGG  
11161 CAAAAGACAA ATATAAGGGT CGAACGAAAA ATAAAGTGAA AAGTGTGAT ATGATGTATT  
11221 TGGCTTTGCG GCGCCGAAAA AACGAGTTTA CGCAATTGCA CAATCATGCT GACTCTGTGG  
11281 CGGACCCGCG CTCTTGCCGG CCCGGCGATA ACGCTGGGCG TGAGGCTGTG CCCGGCGGAG  
11341 TTTTGTGCGC CTGCATTTTC CAAGGTTTAC CCTGCGCTAA GGGGCGAGAT TGGAGAAGCA  
11401 ATAAGAATGC CGGTTGGGGT TGCGATGATG ACGACCACGA CAACTGGTGT CATTATTTAA  
11461 GTTGCCGAAA GAACCTGAGT GCATTTGCAA CATGAGTATA CTAGAAGAAT GAGCCAAGAC  
11521 TTGCGAGACG CGAGTTTGCC GGTGGTGCGA ACAATAGAGC GACCATGACC TTGAAGGTGA  
11581 GACGCGCATA ACCGCTAGAG TACTTTGAAG AGGAAACAGC AATAGGGTTG CTACCAGTAT  
11641 AAATAGACAG GTACATACAA CACTGGAAAT GGTTGTCTGT TTGAGTACGC TTTCAATTCA  
11701 TTTGGGTGTG CAC

FIGURE 415



Figure 42A:

pDEST22

2-Hybrid Vector with  
Activation Domain

657 acg cac act act ctc taa tga gca acg gta tac ggc ctt cct tcc agt tac  
tgc gtg tga tga gag att act cgt tgc cat atg ccg gaa gga agg tca atg

708 ttg aat ttg aaa taa aaa aag ttt gcc gct ttg cta tca agt ata aat aga  
aac tta aac ttt att ttt ttc aaa cgg cga aac gat agt tca tat tta tct

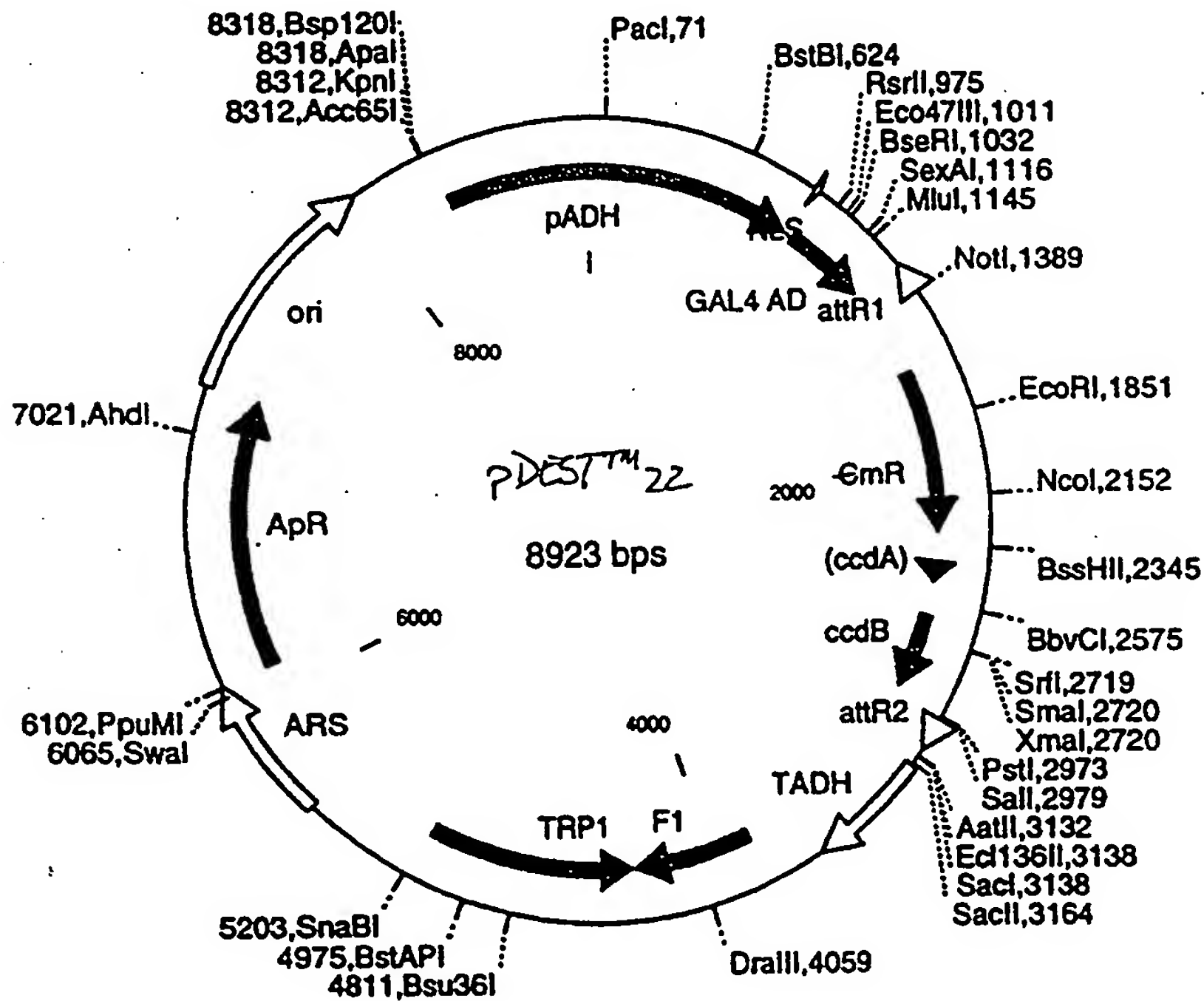
759 cct gca att att aat ctt ttg ttt cct cgt cat tgt tct cgt tcc ctt tct  
gga cgt taa taa tta gaa aac aaa gga gca gta aca aga gca agg gaa aga

810 *ADH Promoter*  
"tcc ttg ttt ctt ttc gtc cat aat att tca agc tat acc aag cat aca atc"  
"agg aac aaa gaa aaa gac gtg tta taa agt tgc ata tgg ttc gta tct tag"

861 *Gal4-AD*  
"aac tcc aag ctt atg ccc aag aag aag cgg aag gtc tgc agc ggc gcc aat"  
"ttg agg ttc gaa tac ggg ttc ttc ttc gcc ttc cag agc tgc ccg cgg tta"  
*Start Translation*

1218 gaa gat acc cca cca aac cca aaa aaa gag ggt ggg tgc aat caa aca agt  
ctt cta tgg ggt ggt ttg ggt ttt ttt ctc cca ccc agc tta gtt tgt tca  
*L Y K K A attR1*

1269 "ttg tac aaa aaa gct gaa cga gaa acg taa a"  
"aac atg ttt ttt cga ctt gct ctt tgc att t"  
*Int*



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## pDEST22 8923 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
904..1248		GAL4 AD
1388..1264		attR1
1638..2297		CmR
2417..2501		inactivated ccdA
2639..2944		ccdB
2985..3109		attR2
3831..4318		f1 (f1 intergenic region)
4334..5176		TRP1
6110..7194		ampR
8344..866		pADH (yeast ADH promoter)

1	TTCATTTGGG	TGTGCACTTT	ATTATGTTAC	AATATGGAAG	GGAACTTTAC	ACTTCTCCTA
61	TGCACATATA	TTAATTAAAG	TCCAATGCTA	GTAGAGAAGG	GGGGTAACAC	CCCTCCGCGC
121	TCCTTTCCGA	TTTTTTTCTA	AACCGTGGAA	TATTTCCGGAT	ATCCTTTTGT	TGTTTCCGGG
181	TGTACAATAT	GGACTTCCTC	TTTTCTGGCA	ACCAAACCCA	TACATCGGGA	TTCCTATAAT
241	ACCTTCGTTG	GTCTCCCTAA	CATGTAGGTG	GCGGAGGGGA	GATATACAAT	AGAACAGATA
301	CCAGACAAGA	CATAATGGGC	TAAACAAGAC	TACACCAATT	ACACTGCCTC	ATTGATGGTG
361	GTACATAACG	AACTAATACT	GTAGCCCTAG	ACTTGATAGC	CATCATCATA	TCGAAGTTTC
421	ACTACCCTTT	TTCCATTTGC	CATCTATTGA	AGTAATAATA	GGCGCATGCA	ACTTCTTTTC
481	TTTTTTTTTC	TTTTCTCTCT	CCCCCGTTGT	TGTCTCACCA	TATCCGCAAT	GACAAAAAAA
541	ATGATGGAAG	ACACTAAAGG	AAAAAATTAA	CGACAAAGAC	AGCACCAACA	GATGTCGTTG
601	TTCCAGAGCT	GATGAGGGGT	ATCTTCGAAC	ACACGAAACT	TTTTCTTCC	TTCATTACAG
661	CACACTACTC	TCTAATGAGC	AACGGTATAC	GGCCTTCCTT	CCAGTTACTT	GAATTTGAAA
721	TAAAAAAGT	TTGCCGCTTT	GCTATCAAGT	ATAAATAGAC	CTGCAATTAT	TAATCTTTTG
781	TTTCCTCGTC	ATTGTTCTCG	TTCCCTTTCT	TCCTTGTTTC	TTTTTCTGCA	CAATATTTCA
841	AGCTATACCA	AGCATACAAT	CAACTCCAAG	CTTATGCCCA	AGAAGAAGCG	GAAGGTCTCG
901	AGCGGCGCCA	ATTTTAATCA	AAGTGGGAAT	ATTGCTGATA	GCTCATTGTC	CTTCACTTTC
961	ACTAACAGTA	GCAACGGTCC	GAACCTCATA	ACAACCTCAA	CAAATTCTCA	AGCGCTTTCA
1021	CAACCAATTG	CCTCCTCTAA	CGTTCATGAT	AACCTTCATGA	ATAATGAAAT	CACGGCTAGT
1081	AAAATTGATG	ATGGTAATAA	TTCAAAACCA	CTGTCACCTG	GTTGGACGGA	CCAAACTGCG
1141	TATAACGCGT	TTGGAATCAC	TACAGGGATG	TTTAATACCA	CTACAATGGA	TGATGTATAT
1201	AACTATCTAT	TCGATGATGA	AGATACCCCA	CCAAACCCAA	AAAAAGAGGG	TGGGTCTGAAT
1261	CAAACAAGTT	TGTACAAAAA	AGCTGAACGA	GAAACGTAAA	ATGATATAAA	TATCAATATA
1321	TTAAATTAGA	TTTTGCATAA	AAAACAGACT	ACATAATACT	GTAAAACACA	ACATATCCAG
1381	TCCTATGGC	GGCCGCTAAG	TTGGCAGCAT	CACCCGACGC	ACTTTGCGCC	GAATAAATAC
1441	CTGTGACGGA	AGATCACTTC	GCAGAATAAA	TAAATCCTGG	TGTCCCTGTT	GATACCGGGA
1501	AGCCCTGGGC	CAACTTTTGG	CGAAATGAG	ACGTTGATCG	GCACGTAAGA	GGTTCCAAC
1561	TTCAACATAA	TGAAATAAGA	TCACTACCGG	GCGTATTTTT	TGAGTTATCG	AGATTTTCAG
1621	GAGCTAAGGA	AGCTAAATG	GAGAAAAAAA	TCACTGGATA	TACCACCGTT	GATATATCCC
1681	AATGGCATCG	TAAAGAACAT	TTTGAGGCAT	TTCAATCAGT	TGCTCAATGT	ACCTATAACC
1741	AGACCGTTCA	GCTGGATATT	ACGGCCTTTT	TAAAGACCGT	AAAGAAAAAT	AAGCACAAGT
1801	TTTATCCGGC	CTTTATTAC	ATTCTTGCCC	GCCTGATGAA	TGCTCATCCG	GAATTCGGTA
1861	TGGCAATGAA	AGACGGTGAG	CTGGTGATAT	GGGATAGTGT	TCACCCTTGT	TACACCGTTT
1921	TCCATGAGCA	AACTGAAACG	TTTTCATCGC	TCTGGAGTGA	ATACCACGAC	GATTTCCGGC
1981	AGTTTCTACA	CATATATTCT	CAAGATGTGG	CGTGTACGG	TGAAAACCTG	GCCTATTTCC
2041	CTAAAGGGTT	TATTGAGAAT	ATGTTTTTCG	TCTCAGCCAA	TCCCTGGGTG	AGTTTCACCA
2101	GTTTGTGATT	AAACGTGGCC	AATATGGACA	ACTTCTTCGC	CCCCGTTTTT	ACCATGGGCA
2161	AATATTATAC	GCAAGGCGAC	AAGGTGCTGA	TGCCGCTGGC	GATTCAGGTT	CATCATGCCG
2221	TCTGTGATGG	CTTCCATGTC	GGCAGAATGC	TTAATGAATT	ACAACAGTAC	TGCGATGAGT
2281	GGCAGGGCGG	GGCGTAATCT	AGAGGATCCG	GCTTACTAAA	AGCCAGATAA	CAGTATGCGT
2341	ATTGCGCGC	TGATTTTTGC	GGTATAAGAA	TATATACTGA	TATGTATACC	CGAAGTATGT
2401	CAAAAAGAGG	TGTGCTATGA	AGCAGCGTAT	TACAGTGACA	GTTGACAGCG	ACAGCTATCA
2461	GTTGCTCAAG	GCATATATGA	TGTCAATATC	TCCGGTCTGG	TAAGCACAAC	CATGCAGAAT
2521	GAAGCCCGTC	GTCTGCGTGC	CGAACGCTGG	AAAGCGGAAA	ATCAGGAAGG	GATGGCTGAG-

FIGURE 42B

2581 GTCGCCCGGT TTATTGAAAT GAACGGCTCT TTTGCTGACG AGAACAGGGA CTGGTGAAAT  
2641 GCAGTTTAAG GTTTACACCT ATAAAAGAGA GAGCCGTTAT CGTCTGTTTG TGGATGTACA  
2701 GAGTGATATT ATTGACACGC CCGGGCGACG GATGGTGATC CCCCTGGCCA GTGCACGTCT  
2761 GCTGTCAGAT AAAGTCTCCC GTGAACTTTA CCCGGTGGTG CATATCGGGG ATGAAAGCTG  
2821 GCGCATGATG ACCACCGATA TGGCCAGTGT GCCGGTCTCC GTTATCGGGG AAGAAGTGGC  
2881 TGATCTCAGC CACCGCGAAA ATGACATCAA AAACGCCATT AACCTGATGT TCTGGGGAAT  
2941 ATAAATGTCA GGCTCCCTTA TACACAGCCA GTCTGCAGGT CGACCATAGT GACTGGATAT  
3001 GTTGTGTTTT ACAGTATTAT GTAGTCTGTT TTTTATGCAA AATCTAATTT AATATATTGA  
3061 TATTTATATC ATTTTACGTT TCTCGTTCAG CTTTCTTGTA CAAAGTGGTT TGATGGCCGC  
3121 TAAGTAAGTA AGACGTCGAG CTCTAAGTAA GTAACGGCCG CCACCGCGGT GGAGCTTTGG  
3181 ACTTCTTCGC CAGAGGTTTG GTCAAGTCTC CAATCAAGGT TGTCCGCTTG TCTACCTTGC  
3241 CAGAAATTTA CGAAAAGATG GAAAAGGGTC AAATCGTTGG TAGATACGTT GTTGACACTT  
3301 CTAAATAAGC GAATTTCTTA TGATTTATGA TTTTATTAT TAAATAAGTT ATAAAAAATA  
3361 TAAGTGATA CAAATTTTAA AGTGACTCTT AGGTTTAAAC ACGAAAATTC TTATTCTTGA  
3421 GTAACCTTTT CCTGTAGGTC AGGTTGCTTT CTCAGGTATA GCATGAGGTC GCTCTTATTG  
3481 ACCACACCTC TACCGGCATG CCGAGCAAAT GCCTGCAAAT CGCTCCCAT TTCACCCAAT  
3541 TGTAGATATG CTAATCCAG CAATGAGTTG ATGAATCTCG GTGTGTATTT TATGTCCTCA  
3601 GAGGACAATA CCTGTTGTAA TCGTTCCTCC ACACGGATCC CAATTCGCCC TATAGTGAGT  
3661 CGTATTACAA TTCACTGGCC GTCGTTTTAC AACGTCGTGA CTGGGAAAAC CCTGGCGTTA  
3721 CCCAACTTAA TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCGTAAT AGCGAAGAGG  
3781 CCCGCACCGA TCGCCCTTCC CAACAGTTGC GCAGCCTGAA TGGCGAATGG ACGCGCCCTG  
3841 TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTACGCGC AGCGTGACCG CTACACTTGC  
3901 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG  
3961 CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG  
4021 GCACCTCGAC CCCAAAAAAC TTGATTAGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG  
4081 ATAGACGGTT TTTGCCCCCT TGACGTTGGA GTCCACGTTT TTTAATAGTG GACTCTTGTT  
4141 CCAAACCTGA ACAACACTCA ACCCTATCTC GGTCTATTCT TTTGATTTAT AAGGGATTTT  
4201 GCCGATTTCT GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT  
4261 TAACAAAATA TTAACGTTTA CAATTTCTTG ATGCGGTATT TTCTCCTTAC GCATCTGTGC  
4321 GGTATTTTCA ACCGCAGGCA AGTGCACAAA CAATACTTAA ATAAATACTA CTCAGTAATA  
4381 ACCTATTTCT TAGCATTTTT GACGAAATTT GCTATTTTGT TAGAGTCTTT TACACCATTT  
4441 GTCTCCACAC CTCCGCTTAC ATCAACACCA ATAACGCCAT TTAATCTAAG CGCATCACCA  
4501 ACATTTTCTG GCGTCAGTCC ACCAGCTAAC ATAAATGTGA AGCTTTCCGG GCTCTCTTGC  
4561 CTTCCAACCC AGTCAGAAAT CGAGTTCCAA TCCAAAAGTT CACCTGTCCC ACCTGCTTCT  
4621 GAATCAAACA AGGGAATAAA CGAATGAGGT TTCTGTGAAG CTGCACTGAG TAGTATGTTG  
4681 CAGTCTTTTG GAAATACGAG TCTTTTAATA ACTGGCAAAC CGAGGAATCT TTGGTATTCT  
4741 TGCCACGACT CATCTCCATG CAGTTGGACG ATATCAATGC CGTAATCATT GACCAGAGCC  
4801 AAAACATCCT CCTTAGGTTG ATTACGAAAC ACGCCAACCA AGTATTTCCG AGTGCCTGAA  
4861 CTATTTTAT ATGCTTTTAC AAGACTTGAA ATTTTCCTTG CAATAACCGG GTCAATTGTT  
4921 CTCTTTCTAT TGGGCACACA TATAATACCC AGCAAGTCAG CATCGGAATC TAGAGCACAT  
4981 TCTGCGGCTT CTGTGCTCTG CAAGCCGCAA ACTTTCACCA ATGGACCAGA ACTACCTGTG  
5041 AAATTAATAA CAGACATACT CCAAGCTGCC TTTGTGTGCT TAATCACGTA TACTCACGTG  
5101 CTCAATAGTC ACCAATGCCC TCCCTCTTGG CCTCTCCTT TTCTTTTTTC GACCGAATTA  
5161 ATTCTTAATC GGCAAAAAA GAAAAGCTCC GGATCAAGAT TGTACGTAAG GTGACAAGCT  
5221 ATTTTCAAT AAAGAATATC TTCCACTACT GCCATCTGGC GTCATAACTG CAAAGTACAC  
5281 ATATATTACG ATGCTGTCTA TTAAATGCTT CCTATATTAT ATATATAGTA ATGTCGTTTA  
5341 TGGTGCACCT TCAGTACAAT CTGCTCTGAT GCCGCATAGT TAAGCCAGCC CCGACACCCG  
5401 CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA  
5461 GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC  
5521 GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTATAGG TTAATGTCAT GATAATAATG  
5581 GTTTCTTAGG ACGGATCGCT TGCCTGTAAC TTACACGCGC CTCGTATCTT TTAATGATGG  
5641 AATAATTTGG GAATTTACTC TGTGTTTATT TATTTTATG TTTTGTATTT GGATTTTAGA  
5701 AAGTAAATAA AGAAGGTAGA AGAGTTACGG AATGAAGAAA AAAAAATAAA CAAAGGTTTA  
5761 AAAAAATTCA ACAAAGAGCG TACTTTACAT ATATATTTAT TAGACAAGAA AAGCAGATTA  
5821 AATAGATATA CATTCGATTA ACGATAAGTA AAATGTAAAA TCACAGGATT TTCGTGTGTG  
5881 GTCTTCTACA CAGACAAGAT GAAACAATTC GGCATTAATA CCTGAGAGCA GGAAGAGCAA  
5941 GATAAAAGGT AGTATTTGTT GGCGATCCCC CTAGAGTCTT TTACATCTTC GGAAACAAA  
6001 AACTATTTTT TCTTTAATTT CTTTTTTTAC TTTCTATTTT TAATTTATAT ATTTATATTA-

FIGURE 42C

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6061 AAAAATTTAA ATTATAATTA TTTTATAGC ACGTGATGAA AAGGACCCAG GTGGCACTTT  
6121 TCGGGGAAAT GTGCGCGGAA CCCCTATTTG TTTATTTTTC TAAATACATT CAAATATGTA  
6181 TCCGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGTAT  
6241 GAGTATTCAA CATTTCCTGT TCGCCCTTAT TCCCTTTTTT GCGGCATTTT GCCTTCCTGT  
6301 TTTTGCTCAC CCAGAAACGC TGGTGAAAGT AAAAGATGCT GAAGATCAGT TGGGTGCACG  
6361 AGTGGGTAC ATCGAACTGG ATCTCAACAG CGGTAAGATC CTTGAGAGTT TTCGCCCCGA  
6421 AGAACGTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA TGTGGCGCGG TATTATCCCG  
6481 TATTGACGCC GGGCAAGAGC AACTCGGTGC CCGCATACAC TATTCTCAGA ATGACTTGGT  
6541 TGAGTACTCA CCAGTCACAG AAAAGCATCT TACGGATGGC ATGACAGTAA GAGAATTATG  
6601 CAGTGCTGCC ATAACCATGA GTGATAACAC TGCGGCCAAC TTACTTCTGA CAACGATCCG  
6661 AGGACCGAAG GAGCTAACCG CTTTTTTTCA CAACATGGGG GATCATGTAA CTCGCCTTGA  
6721 TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC GAGCGTGACA CCACGATGCC  
6781 TGTAGCAATG GCAACAACGT TGCGCAAAC ATTAAGTGGC GAACTACTTA CTCTAGCTTC  
6841 CCGGCAACAA TTAATAGACT GGATGGAGGC GGATAAAGTT GCAGGACCAC TTCTGCGCTC  
6901 GGCCCTTCCG GCTGGCTGGT TTATTGCTGA TAAATCTGGA GCCGGTGAGC GTGGGTCTCG  
6961 CGGTATCATT GCAGCACTGG GGCCAGATGG TAAGCCCTCC CGTATCGTAG TTATCTACAC  
7021 GACGGGCAGT CAGGCAACTA TGGATGAACG AAATAGACAG ATCGCTGAGA TAGGTGCCTC  
7081 ACTGATTAAG CATTGGTAAC TGTCAGACCA AGTTTACTCA TATATACTTT AGATTGATTT  
7141 AAAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC  
7201 CAAAATCCCT TAACGTGAGT TTTCTGTTCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA  
7261 AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA CAAAAAACC  
7321 ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT  
7381 AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCTT CTAGTGTAGC CGTAGTTAGG  
7441 CCACCACTTC AAGAACTCTG TAGCACCGCC TACATACCTC GCTCTGCTAA TCCTGTTACC  
7501 AGTGGCTGCT GCCAGTGGCG ATAAGTCTGT TCTTACCGGG TTGGACTCAA GACGATAGTT  
7561 ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCC TGACACACAGC CCAGCTTGA  
7621 GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CATTGAGAAA GCGCCACGCT  
7681 TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG  
7741 CACGAGGGAG CTTCAGGGG GGAACGCCTG GTATCTTTAT AGTCCTGTCC GGTTCGCCA  
7801 CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCCGAGCC TATGGAAAAA  
7861 CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT  
7921 CTTTCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCTTTG AGTGAGCTGA  
7981 TACCGCTCGC CGCAGCCGAA CGACCGAGCG CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA  
8041 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA  
8101 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTACCT  
8161 CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCCTATGT TGTGTGGAAT  
8221 TGTGAGCGGA TAACAATTTT ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTCGG  
8281 AATTAACCTT CACTAAAGGG AAAAAAGCT GGGTACCGGG CCCCCCTCG AGATCCGGGA  
8341 TCGAAGAAAT GATGGTAAAT GAAATAGGAA ATCAAGGAGC ATGAAGGCAA AAGACAAATA  
8401 TAAGGGTCGA ACGAAAAATA AAGTGAAAAG TGTTGATATG ATGTATTTGG CTTTGCGGCG  
8461 CCGAAAAAAC GAGTTTACGC AATTGCACAA TCATGCTGAC TCTGTGGCGG ACCCGCGCTC  
8521 TTGCCGGCCC GCGGATAACG CTGGGCGTGA GGCTGTGCCC GGCGGAGTTT TTTGCGCCTG  
8581 CATTTTCCAA GGTTTACCCT GCGCTAAGGG GCGAGATTGG AGAAGCAATA AGAATGCCGG  
8641 TTGGGGTTGC GATGATGACG ACCACGACAA CTGGTGTCTAT TATTTAAGTT GCCGAAAGAA  
8701 CCTGAGTGCA TTTGCAACAT GAGTATACTA GAAGAATGAG CCAAGACTTG CGAGACGCGA  
8761 GTTTGCCGGT GGTGCGAACA ATAGAGCGAC CATGACCTTG AAGGTGAGAC GCGCATAACC  
8821 GCTAGAGTAC TTTGAAGAGG AAACAGCAAT AGGGTTGCTA CCAGTATAAA TAGACAGGTA  
8881 CATAACAC TGGAAATGGT TGTCTGTTT AGTACGCTTT CAA

Figure 42d



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pDEST23

His6 carboxy-fusion vector, T7 promoter,

205    atc ccg cga aat taa tac gac tca cta tag gga gat cac aac ggt ttc cct MRNA  
      tag ggc gct tta att atg ctg agt gat atc cgt ctg gtg ttg cca aag gga  
 256    cta gat aac aag ttt gta caa aaa agc tga acg aga aac gta aaa tga tat att R1  
      gat cta gtg ttc aaa cat gtt ttt tgc act tgc tct ttg cat ttt act ata

// ————— *Cm<sup>R</sup>* ————— *ccd B* ————— //

1888    ttt tta tgc aaa atc taa ttt aat ata ttg ata ttt ata tca ttt taa gtt  
      aaa aat acg ttt tag att aaa tta tat aac tat aaa tat agt aaa atg caa  
 1939    att R2    A F L Y K V Y I M S Y Y H H  
      tct cgt tca gct ttt ttg tac aaa gtg gtg att atg tgc tac tac cat cac  
      aga gca agt cga aag aac atg ttt cac cac taa tac agc atg atg gta gtg  
 1990    H H H H L D E V Q term HIS6  
      cat cac cat cac ctc gat gag caa tag cta gca taa ccc ctt ggg ggc tct  
      gta gtg gta gtg gag cta ctc gtt att gat cgt att ggg gaa ccc cgg aga

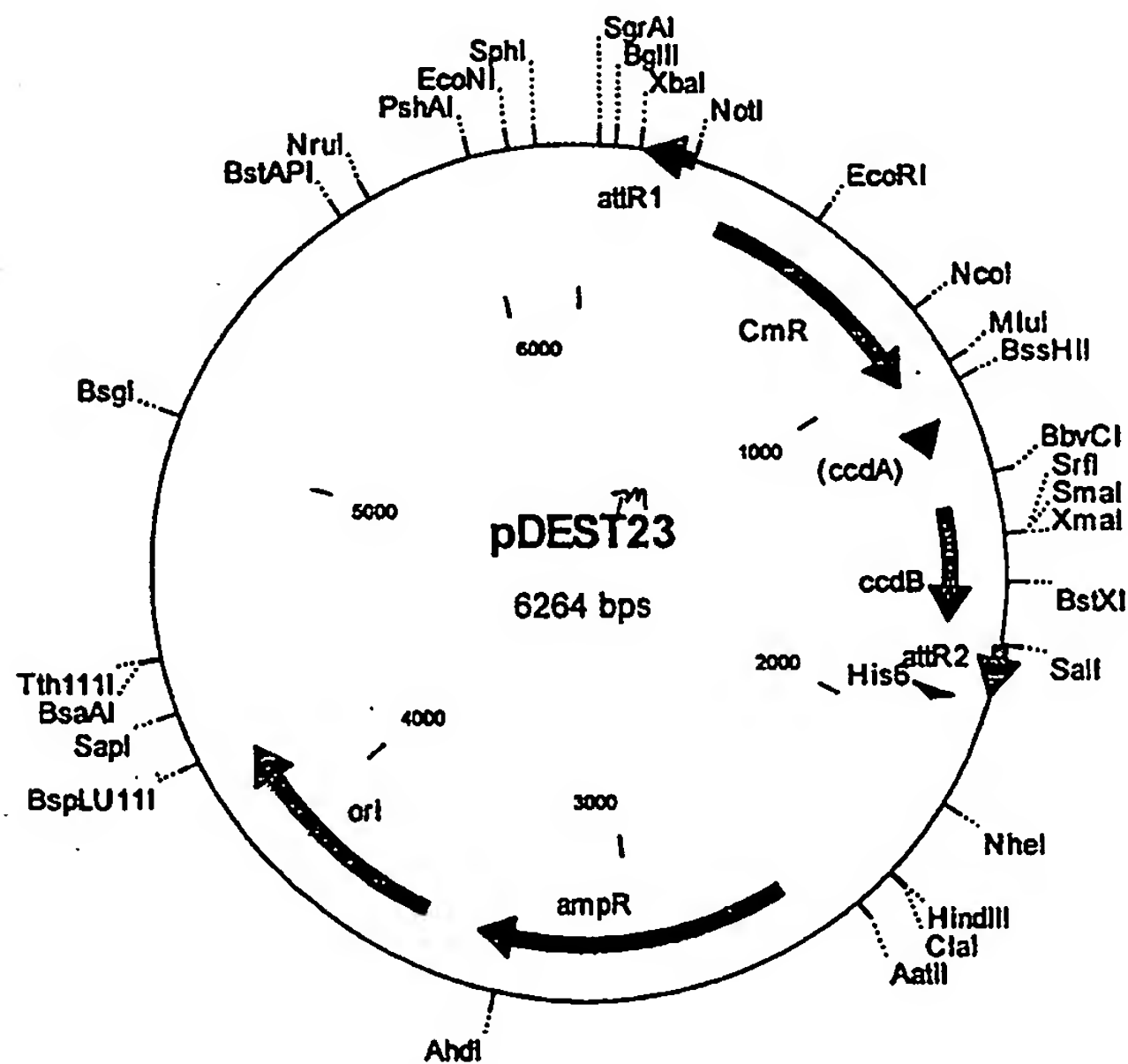


FIGURE 43A

## pDEST23 6264 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
285..161		attR1
394..1053		CmR
1173..1257		inactivated ccdA
1395..1700		ccdB
1741..1865		attR2
1883..1911		his6
2574..3434		ampR
3583..4222		ori
1	TCTTCCCAT CCGTGATGTC GCGGATATAG GCGCCAGCAA CCGCACCTGT GGCGCCGGTG	
61	ATGCCGGCCA CGATGCGTCC GCGGTAGAGG ATCGAGATCT CGATCCCGCG AAATTAATAC	
121	GACTCACTAT AGGGAGACCA CAACGGTTTC CCTCTAGATC ACAAGTTTGT AAAAAAAGC	
181	TGAACGAGAA ACGTAAAATG ATATAAATAT CAATATATTA AATTAGATTT TGCATAAAAA	
241	ACAGACTACA TAATACTGTA AAACACAACA TATCCAGTCA CTATGGCGGC CGCATTAGGC	
301	ACCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATAATG TGTGGATTTT GAGTTAGGAT	
361	CCGGCGAGAT TTTCAGGAGC TAAGGAAGCT AAAATGGAGA AAAAAATCAC TGGATATACC	
421	ACCGTTGATA TATCCCAATG GCATCGTAAA GAACATTTTG AGGCATTTCA GTCAGTTGCT	
481	CAATGTACCT ATAACCAGAC CGTTCAGCTG GATATTACGG CCTTTTAAA GACCGTAAAG	
541	AAAAATAAGC ACAAGTTTAA TCCGGCCTTT ATTCACATTC TTGCCCCCCT GATGAATGCT	
601	CATCCGGAAT TCCGTATGGC AATGAAAGAC GGTGAGCTGG TGATATGGGA TAGTGTTTAC	
661	CCTTGTTACA CCGTTTTCCA TGAGCAAAC GAAACGTTTT CATCGCTCTG GAGTGAATAC	
721	CACGACGATT TCCGGCAGTT TCTACACATA TATTCGCAAG ATGTGGCGTG TTACGGTGAA	
781	AACCTGGCCT ATTTCCCTAA AGGGTTTATT GAGAATATGT TTTTCGTCTC AGCCAATCCC	
841	TGGGTGAGTT TCACCAGTTT TGATTTAAAC GTGGCCAATA TGGACAACCT CTTGCCCCC	
901	GTTTTACCA TGGGCAAATA TTATACGCAA GCGACAAGG TGCTGATGCC GCTGGCGATT	
961	CAGGTTTCATC ATGCCGTCTG TGATGGCTTC CATGTCGGCA GAATGCTTAA TGAATTACAA	
1021	CAGTACTGCG ATGAGTGGCA GGGCGGGGCG TAAACGCGTG GATCCGGCTT ACTAAAAGCC	
1081	AGATAACAGT ATGCGTATTT GCGCGCTGAT TTTTGCGGTA TAAGAATATA TACTGATATG	
1141	TATACCCGAA GTATGTCAAA AAGAGGTGTG CTATGAAGCA GCGTATTACA GTGACAGTTG	
1201	ACAGCGACAG CTATCAGTTG CTCAAGGCAT ATATGATGTC AATATCTCCG GTCTGGTAAG	
1261	CACAACCATG CAGAATGAAG CCCGTCGTCT GCGTGCCGAA CGCTGGAAAG CGGAAAATCA	
1321	GGAAGGGATG GCTGAGGTCG CCCGGTTTAT TGAAATGAAC GGCTCTTTTG CTGACGAGAA	
1381	CAGGGACTGG TGAAATGCAG TTAAAGGTTT ACACCTATAA AAGAGAGAGC CGTTATCGTC	
1441	TGTTTGTGGA TGTACAGAGT GATATTATTG ACACGCCCGG GCGACGGATG GTGATCCCCC	
1501	TGGCCAGTGC ACGTCTGCTG TCAGATAAAG TCTCCCGTGA ACTTTACCCG GTGGTGATA	
1561	TCGGGGATGA AAGCTGGCGC ATGATGACCA CCGATATGGC CAGTGTGCCG GTCTCCGTTA	
1621	TCGGGGAAGA AGTGGCTGAT CTCAGCCACC GCGAAAATGA CATCAAAAC GCCATTAAAC	
1681	TGATGTTCTG GGGAAATATA ATGTCAGGCT CCCTTATACA CAGCCAGTCT GCAGGTCGAC	
1741	CATAGTGAAT GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC	
1801	TAATTTAATA TATTGATATT TATATCATT TACGTTTCTC GTTCAGCTTT CTTGTACAAA	
1861	GTGGTGATTA TGTCGTACTA CCATCACCAT CACCATCACC TCGATGAGCA ATAAGTAGCA	
1921	TAACCCCTTG GGGCCTCTAA ACGGGTCTTG AGGGGTTTTT TGCTGAAAGG AGGAACTATA	
1981	TCCGGATATC CACAGGACGG GTGTGGTCGC CATGATCGCG TAGTCGATAG TGGCTCCAAG	
2041	TAGCGAAGCG AGCAGGACTG GCGGGCGGCC AAAGCGGTCG GACAGTGCTC CGAGAACGGG	
2101	TGCGCATAGA AATTGCATCA ACGCATATAG CGCTAGCAGC ACGCCATAGT GACTGGCGAT	
2161	GCTGTCGGAA TGGACGATAT CCCGCAAGAG GCCCGGCAGT ACCGGCATAA CCAAGCCTAT	
2221	GCCTACAGCA TCCAGGGTGA CCGTGCCGAG GATGACGATG AGCGCATTGT TAGATTTTAT	
2281	ACACGGTGCC TGACTGCGTT AGCAATTAA CTGTGATAAA CTACCGCATT AAAGCTTATC	
2341	GATGATAAGC TGTCAAACAT GAGAATTCTT GAAGACGAAA GGGCCTCGTG ATACGCCTAT	
2401	TTTTATAGGT TAATGTCATG ATAATAATGG TTTCTTAGAC GTCAGGTGGC ACTTTTCGGG	
2461	GAAATGTGCG CGGAACCCCT ATTTGTTTAT TTTTCTAAAT ACATTCAAAT ATGTATCCGC	
2521	TCATGAGACA ATAACCCTGA TAAATGCTTC AATAATATTG AAAAAGGAAG AGTATGAGTA	
2581	TTCAACATTT CCGTGTGCGC CTTATTCCCT TTTTTCGGC ATTTTGCCTT CCTGTTTTTG	
2641	CTCACCAGAA AACGCTGGTG AAAGTAAAG ATGCTGAAGA TCAGTTGGGT GCACGAGTGG-	

FIGURE 43B



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2701 GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAC  
2761 GTTTTCCAAT GATGAGCACT TTTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG  
2821 ACGCCGGGCA AGAGCAACTC GGTCGCCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT  
2881 ACTCACCAGT CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG  
2941 CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC  
3001 CGAAGGAGCT AACCCTTTT TTGCACAACA TGGGGGATCA TGTAACCTCGC CTTGATCGTT  
3061 GGAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCACG ATGCCTGCAG  
3121 CAATGGCAAC AACGTTGCGC AAATATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC  
3181 AACAATTAAT AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC  
3241 TTCCGGCTGG CTGGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA  
3301 TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG  
3361 GGAGTCAGGC AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA  
3421 TTAAGCATTG GTAACGTGCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTAAAAC  
3481 TTCATTTTAA ATTTAAAAGG ATCTAGGTGA AGATCCTTTT TGATAATCTC ATGACCAAAA  
3541 TCCCTTAACG TGAGTTTTTCG TTCCACTGAG CGTCAGACCC CGTAGAAAAG ATCAAAGGAT  
3601 CTTCTTGAGA TCCTTTTTTTT CTGCGCGTAA TCTGCTGCTT GCAAACAAAA AAACCACCGC  
3661 TACCAGCGGT GGTGTTGTTG CCGGATCAAG AGCTACCAAC TCTTTTTCCG AAGGTAAGTG  
3721 GCTTCAGCAG AGCGCAGATA CCAAATACTG TCCTTCTAGT GTAGCCGTAG TTAGGCCACC  
3781 ACTTCAAGAA CTCTGTAGCA CCGCCTACAT ACCTCGCTCT GCTAATCCTG TTACCAGTGG  
3841 CTGCTGCCAG TGGCGATAAG TCGTGTCTTA CCGGGTTGGA CTCAAGACGA TAGTTACCGG  
3901 ATAAGGCGCA GCGGTCGGGC TGAACGGGGG GTTCGTGCAC ACAGCCCAGC TTGGAGCGAA  
3961 CGACCTACAC CGAACTGAGA TACCTACAGC GTGAGCTATG AGAAAGCGCC ACGCTTCCCG  
4021 AAGGGAGAAA GCGGACAGG TATCCGGTAA GCGGCAGGGT CGGAACAGGA GAGCGCACGA  
4081 GGGAGCTTCC AGGGGGAAAC GCCTGGTATC TTTATAGTCC TGTCGGGTTT CGCCACCTCT  
4141 GACTTGAGCG TCGATTTTTG TGATGCTCGT CAGGGGGGCG GAGCCTATGG AAAAAGCCCA  
4201 GCAACGCGGC CTTTTTACGG TTCCTGGCCT TTTGCTGGCC TTTTGCTCAC ATGTTCTTTC  
4261 CTGCGTTATC CCTGATTCT GTGGATAACC GTATTACCGC CTTTGAGTGA GCTGATACCG  
4321 CTCGCCGAG CCGAACGACC GAGCGCAGCG AGTCAGTGAG CGAGGAAGCG GAAGAGCGCC  
4381 TGATGCGGTA TTTTCTCCTT ACGCATCTGT GCGGTATTTT ACACCGCATA TATGGTGCAC  
4441 TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATACACTCC GCTATCGCTA  
4501 CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG  
4561 GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG  
4621 TGTCAGAGGT TTTACCGTC ATCACCAGAA CGCGCGAGGC AGCTGCGGTA AAGCTCATCA  
4681 GCGTGGTCGT GAAGCGATTC ACAGATGTCT GCCTGTTTCT CCGCGTCCAG CTCGTTGAGT  
4741 TTCTCCAGAA GCGTTAATGT CTGGCTTCTG ATAAAGCGGG CCATGTTAAG GCGGTTTTT  
4801 TCCTGTTTGG TCACTGATGC CTCCGTGTAA GGGGGATTTT TGTTTCATGGG GGTAATGATA  
4861 CCGATGAAAC GAGAGAGGAT GCTCACGATA CCGGTTACTG ATGATGAACA TGCCCGGTTA  
4921 CTGGAACGTT GTGAGGGTAA ACAACTGGCG GTATGGATGC GGCGGGACCA GAGAAAAATC  
4981 ACTCAGGGTC AATGCCAGCG CTTCTGTTAAT ACAGATGTAG GTGTTCCACA GGGTAGCCAG  
5041 CAGCATCCTG CGATGCAGAT CCGGAACATA ATGGTGCAGG GCGCTGACTT CCGCGTTTCC  
5101 AGACTTTACG AAACACGGAA ACCGAAGACC ATTCATGTTG TTGCTCAGGT CGCAGACGTT  
5161 TTGCAGCAGC AGTCGCTTCA CGTTCGCTCG CGTATCGGTG ATTCATTCTG CTAACCAGTA  
5221 AGGCAACCCC GCCAGCCTAG CCGGGTCCTC AACGACAGGA GCACGATCAT GCGCACCCTG  
5281 GGCCAGGACC CAACGCTGCC CGAGATGCGC CGCGTGCGGC TGCTGGAGAT GGCGGACGCG  
5341 ATGGATATGT TCTGCCAAGG GTTGGTTTTG GCATTCACAG TTCTCCGCAA GAATTGATTG  
5401 GCTCCAATTC TTGGAGTGGT GAATCCGTTA GCGAGGTGCC GCCGGCTTCC ATTCAGGTG  
5461 AGGTGGCCCC GCTCCATGCA CCGCGACGCA ACGCGGGGAG GCAGACAAGG TATAGGGCGG  
5521 CGCCTACAA CCATGCCAAC CCGTTCCATG TGCTCGCCGA GGCGGCATAA ATCGCCGTGA  
5581 CGATCAGCGG TCCAGTGATC GAAGTTAGGC TGGTAAGAGC CGCGAGCGAT CCTTGAAGCT  
5641 GTCCCTGATG GTCGTCATCT ACCTGCCTGG ACAGCATGGC CTGCAACGCG GGCATCCCGA  
5701 TGCCGCCGGA AGCGAGAAGA ATCATAATGG GGAAGGCCAT CCAGCCTCGC GTCGCGAAGC  
5761 CCAGCAAGAC GTAGCCCAGC GCGTCGGCCG CCATGCCGGC GATAATGGCC TGCTTCTCGC  
5821 CGAAACGTTT GGTGGCGGGA CCAGTGACGA AGGCTTGAGC GAGGGCGTGC AAGATTCCGA  
5881 ATACCGCAAG CGACAGGCCG ATCATCGTCG CGCTCCAGCG AAAGCGGTCC TCGCCGAAAA  
5941 TGACCCAGAG CGCTGCCGGC ACCTGTCCTA CGAGTTGCAT GATAAAGAAG ACAGTCATAA  
6001 GTGCGGCGAC GATAGTCATG CCCCAGCGCC ACCGGAAGGA GCTGACTGGG TTGAAGGCTC  
6061 TCAAGGGCAT CCGTCGATCG ACGCTCTCCC TTATGCGACT CCTGCATTAG GAAGCAGCCC  
6121 AGTAGTAGGT TGAGGCCGTT GAGCACCACC GCCGCAAGGA ATGGTGCATG CAAGGAGATG-

FIGURE 43C

WO 00/52027

PCT/US00/05432

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6181 GCGCCCAACA GTCCCCCGGC CACGGGGCCT GCCACCATAC CCACGCCGAA ACAAGCGCTC  
6241 ATGAGCCCGA AGTGGCGAGC CCGA

FIGURE 43D

PDEST24

11 ————— c m R ————— c c d B ————— 11

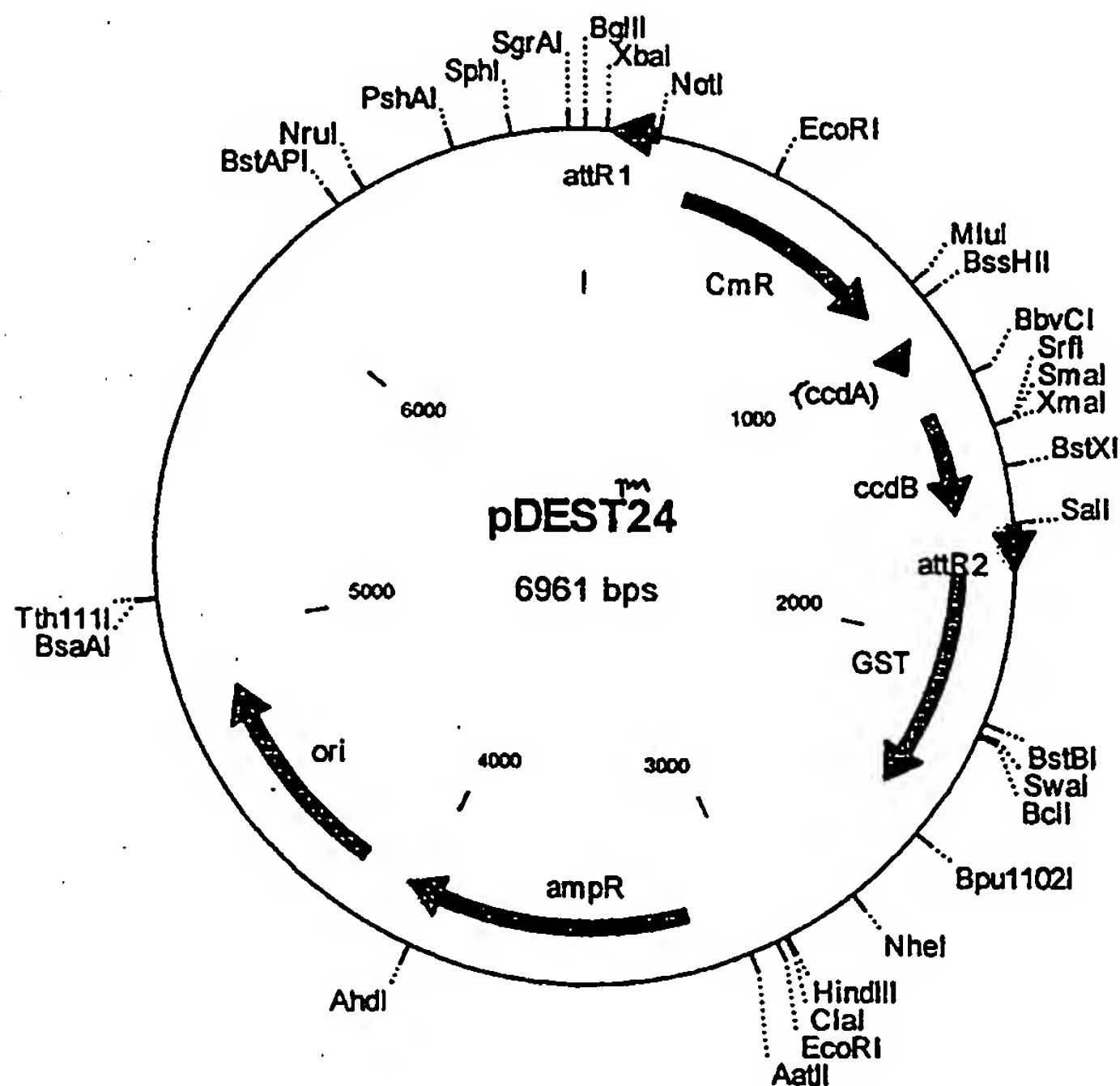


FIGURE 44A

125/240

pDEST24 6961 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
195..71		attR1
304..963		CmR
1083..1167		inactivated ccdA
1305..1610		ccdB
1651..1775		attR2
1783..2451		GST
3181..4041		ampR
4190..4829		ori

1	ATCGAGATCT	CGATCCCGCG	AAATTAATAC	GACTCACTAT	AGGGAGACCA	CAACGGTTTC
61	CCTCTAGATC	ACAAGTTTGT	ACAAAAAGC	TGAACGAGAA	ACGTAAAATG	ATATAAATAT
121	CAATATATTA	AATTAGATTT	TGCATAAAAA	ACAGACTACA	TAATACTGTA	AAACACAACA
181	TATCCAGTCA	CTATGGCGGC	CGCATTAGGC	ACCCAGGCT	TTACACTTTA	TGCTTCCGGC
241	TCGTATAATG	TGTGGATTTT	GAGTTAGGAT	CCGGCGAGAT	TTTCAGGAGC	TAAGGAAGCT
301	AAAATGGAGA	AAAAAATCAC	TGGATATACC	ACCGTTGATA	TATCCCAATG	GCATCGTAAA
361	GAACATTTTG	AGGCATTTCA	GTCAGTTGCT	CAATGTACCT	ATAACCAGAC	CGTTCAGCTG
421	GATATTACGG	CCTTTTAAA	GACCGTAAAG	AAAAATAAGC	ACAAGTTTAA	TCCGGCCTTT
481	ATTCACATTC	TTGCCCGCCT	GATGAATGCT	CATCCGGAAT	TCCGTATGGC	AATGAAAGAC
541	GGTGAGCTGG	TGATATGGGA	TAGTGTTCAC	CCTTGTTACA	CCGTTTTCCA	TGAGCAAAC
601	GAAACGTTTT	CATCGCTCTG	GAGTGAATAC	CACGACGATT	TCCGGCAGTT	TCTACACATA
661	TATTCGCAAG	ATGTGGCGTG	TTACGGTGAA	AACCTGGCCT	ATTTCCCTAA	AGGGTTTATT
721	GAGAATATGT	TTTTCGTCTC	AGCCAATCCC	TGGGTGAGTT	TCACCAGTTT	TGATTTAAAC
781	GTGGCCAATA	TGGACAACCT	CTTCGCCCCC	GTTTTACCA	TGGGCAAATA	TTATACGCAA
841	GGCGACAAGG	TGCTGATGCC	GCTGGCGATT	CAGGTTTCATC	ATGCCGCTCG	TGATGGCTTC
901	CATGTCGGCA	GAATGCTTAA	TGAATTACAA	CAGTACTGCG	ATGAGTGGCA	GGGCGGGGCG
961	TAAACGCGTG	GATCCGGCTT	ACTAAAAGCC	AGATAACAGT	ATGCGTATTT	GCGCGCTGAT
1021	TTTTGCGGTA	TAAGAATATA	TACTGATATG	TATACCCGAA	GTATGTCAAA	AAGAGGTGTG
1081	CTATGAAGCA	GCGTATTACA	GTGACAGTTG	ACAGCGACAG	CTATCAGTTG	CTCAAGGCAT
1141	ATATGATGTC	AATATCTCCG	GTCTGGTAAG	CACAACCATG	CAGAATGAAG	CCCGTCGTCT
1201	GCGTGCCGAA	CGCTGGAAAG	CGGAAAATCA	GGAAGGGATG	GCTGAGGTCG	CCCGGTTTAT
1261	TGAAATGAAC	GGCTCTTTTG	CTGACGAGAA	CAGGGACTGG	TGAAATGCAG	TTTAAGGTTT
1321	ACACCTATAA	AAGAGAGAGC	CGTTATCGTC	TGTTTGTGGA	TGTACAGAGT	GATATTATTG
1381	ACACGCCCCG	GCGACGGATG	GTGATCCCCC	TGGCCAGTGC	ACGTCTGCTG	TCAGATAAAG
1441	TCTCCCGTGA	ACTTTACCCG	GTGGTGATA	TCGGGGATGA	AAGCTGGCGC	ATGATGACCA
1501	CCGATATGGC	CAGTGTGCCG	GTCTCCGTTA	TCGGGGAAGA	AGTGGCTGAT	CTCAGCCACC
1561	GCGAAAATGA	CATCAAAAAC	GCCATTAACC	TGATGTTCTG	GGGAATATAA	ATGTCAGGCT
1621	CCCTTATACA	CAGCCAGTCT	GCAGGTCGAC	CATAGTGA	GGATATGTTG	TGTTTTACAG
1681	TATTATGTAG	TCTGTTTTTT	ATGCAAAATC	TAATTTAATA	TATTGATATT	TATATCATTT
1741	TACGTTTCTC	GTTTCTTTT	CTTGACAAA	GTGGTGATTA	TGTCCCCTAT	ACTAGGTTAT
1801	TGGAAAATTA	AGGGCCTTGT	GCAACCCACT	CGACTTCTTT	TGGAATATCT	TGAAGAAAAA
1861	TATGAAGAGC	ATTTGTATGA	GCGCGATGAA	GGTGATAAAT	GGCGAAACAA	AAAGTTTGAA
1921	TTGGGTTTGG	AGTTTCCCAA	TCTTCCTTAT	TATATTGATG	GTGATGTTAA	ATTAACACAG
1981	TCTATGGCCA	TCATACGTTA	TATAGCTGAC	AAGCACAACA	TGTTGGGTGG	TTGTCCAAAA
2041	GAGCGTGCAG	AGATTTCAAT	GCTTGAAGGA	GCGGTTTTGG	ATATTAGATA	CGGTGTTTCG
2101	AGAATTGCAT	ATAGTAAAGA	CTTTGAAACT	CTCAAAGTTG	ATTTTCTTAG	CAAGCTACCT
2161	GAAATGCTGA	AAATGTTTGA	AGATCGTTTA	TGTCATAAAA	CATATTTAAA	TGGTGATCAT
2221	GTAACCCATC	CTGACTTCAT	GTTGTATGAC	GCTCTTGATG	TTGTTTTATA	CATGGACCCA
2281	ATGTGCCTGG	ATGCGTTCCC	AAAATTAGTT	TGTTTTAAAA	AACGTATTGA	AGCTATCCCA
2341	CAAATTGATA	AGTACTTGAA	ATCCAGCAAG	TATATAGCAT	GGCCTTTGCA	GGGCTGGCAA
2401	GCCACGTTTG	GTGGTGCGCA	CCATCCTCCA	AAATCGGATC	TGGTTCCGCG	TCCATGGGGA
2461	TCCGGCTGCT	AACAAAGCCC	GAAAGGAAGC	TGAGTTGGCT	GCTGCCACCG	CTGAGCAATA
2521	ACTAGCATAA	CCCCTTGGGG	CCTCTAAACG	GGTCTTGAGG	GGTTTTTTGC	TGAAAGGAGG
2581	AACTATATCC	GGATATCCAC	AGGACGGGTG	TGGTCGCCAT	GATCGCGTAG	TCGATAGTGG
2641	CTCCAAGTAG	CGAAGCGAGC	AGGACTGGGC	GGCGGCCAAA	GCGGTCGGAC	AGTGCTCCGA-

FIGURE 44B



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2701 GAACGGGTGC GCATAGAAAT TGCATCAACG CATATAGCGC TAGCAGCAGC CCATAGTGAC  
2761 TGGCGATGCT GTCGGAATGG ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA  
2821 AGCCTATGCC TACAGCATCC AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG  
2881 ATTTCATACA CGGTGCCTGA CTGCGTTAGC AATTCTTAACTG TGATAAACTA CCGCATTAAA  
2941 GCTTATCGAT GATAAGCTGT CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA  
3001 CGCCTATTTT TATAGGTTAA TGTCTATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT  
3061 TTTCGGGGAA ATGTGCGCGG AACCCTATT TGTCTATTTT TCTAAATACA TTCAAATATG  
3121 TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT  
3181 ATGAGTATTC AACATTTCCG TGTGCGCCCTT ATTCCTTTT TTGCGGCATT TTGCCTTCCT  
3241 GTTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA  
3301 CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC  
3361 GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTCTGTC TATGTGGCGC GGTATTATCC  
3421 CGTGTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG  
3481 GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA  
3541 TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC  
3601 GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT  
3661 GATCGTTGGG AACCAGGAGCT GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG  
3721 CCTGCAGCAA TGGCAACAAC GTTGCAGCAA CTATTAAGT GCGAACTACT TACTCTAGCT  
3781 TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC  
3841 TCGGCCCTTC CGGCTGGCTG GTTTATGTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT  
3901 CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC  
3961 ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC  
4021 TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT  
4081 TTAAACTTC ATTTTAAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG  
4141 ACCAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC  
4201 AAAGGATCTT CTTGAGATCC TTTTCTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAA  
4261 CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG  
4321 GTAAGTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGA GCCGTAGTTA  
4381 GGCCACCACT TCAAGAACTC TGTAGCACC GCTACATACC TCGCTCTGCT AATCCTGTTA  
4441 CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC AAGACGATAG  
4501 TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACACA GCCCAGCTTG  
4561 GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG  
4621 CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG  
4681 CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT CCGGTTTCGC  
4741 CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA  
4801 AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG  
4861 TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT  
4921 GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA  
4981 GAGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT  
5041 GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACTCCGCT  
5101 ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC  
5161 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG  
5221 CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC GCGAGGCAGC TGCGGTAAAG  
5281 CTCATCAGCG TGGTCGTGAA GCGATTACA GATGTCTGCC GTTTCATCCG CGTCCAGCTC  
5341 GTTGAGTTTC TCCAGAAGCG TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC  
5401 GGTTTTTTCC TGTTTGGTCA CTGATGCCTC CGTGTAAGGG GGATTTCTGT TCATGGGGGT  
5461 AATGATACCG ATGAAACGAG AGAGGATGCT CACGATACGG GTTACTGATG ATGAACATGC  
5521 CCGGTTACTG GAACGTTGTG AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG  
5581 AAAAATCACT CAGGGTCAAT GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG  
5641 TAGCCAGCAG CATCTGCGA TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG  
5701 CGTTTCCAGA CTTTACGAAA CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGGTCCG  
5761 AGACGTTTTG CAGCAGCAGT CGCTTCACGT TCGCTCGCGT ATCGGTGATT CATTCTGCTA  
5821 ACCAGTAAGG CAACCCCGCC AGCCTAGCCG GGTCCTCAAC GACAGGAGCA CGATCATGCG  
5881 CACCGTGCGC CAGGACCCAA CGCTGCCCCG GATGCGCCGC GTGCGGCTGC TGGAGATGGC  
5941 GGACGCGATG GATATGTTCT GCCAAGGGTT GGTTTGCBCA TTCACAGTTC TCCGCAAGAA  
6001 TTGATTGGCT CCAATTCTTG GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT  
6061 CAGGTCGAGG TGGCCCGGCT CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT  
6121 AGGGCGGCGC CTACAATCCA TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC

FIGURE 44C

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6181 GCCGTGACGA TCAGCGGTCC AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT  
6241 TGAAGCTGTC CCTGATGGTC GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC  
6301 ATCCCGATGC CGCCGGAAGC GAGAAGAATC ATAATGGGGA AGGCCATCCA GCCTCGCGTC  
6361 GCGAACGCCA GCAAGACGTA GCCCAGCGCG TCGGCCGCCA TGCCGGCGAT AATGGCCTGC  
6421 TTCTCGCCGA AACGTTTGGT GGCGGGACCA GTGACGAAGG CTTGAGCGAG GGCCTGCAAG  
6481 ATTCCGAATA CCGCAAGCGA CAGGCCGATC ATCGTCGCGC TCCAGCGAAA GCGGTCCTCG  
6541 CCGAAAATGA CCCAGAGCGC TGCCGGCACC TGTCTACGA GTTGATGAT AAAGAAGACA  
6601 GTCATAAGTG CGGCGACGAT AGTCATGCCC CGCGCCCACC GGAAGGAGCT GACTGGGTTG  
6661 AAGGCTCTCA AGGGCATCGG TCGATCGACG CTCTCCCTTA TGCGACTCCT GCATTAGGAA  
6721 GCAGCCCAGT AGTAGGTTGA GGCCGTTGAG CACCGCCGCC GCAAGGAATG GTGCATGCAA  
6781 GGAGATGGCG CCCAACAGTC CCCCGGCCAC GGGGCCTGCC ACCATACCCA CGCCGAAACA  
6841 AGCGCTCATG AGCCCGAAGT GGCAGAGCCG ATCTTCCCA TCGGTGATGT CGGCGATATA  
6901 GGCGCCAGCA ACCGCACCTG TGGCGCCGCT GATGCCGGCC ACGATGCGTC CGGCGTAGAG  
6961 G

FIGURE 44D

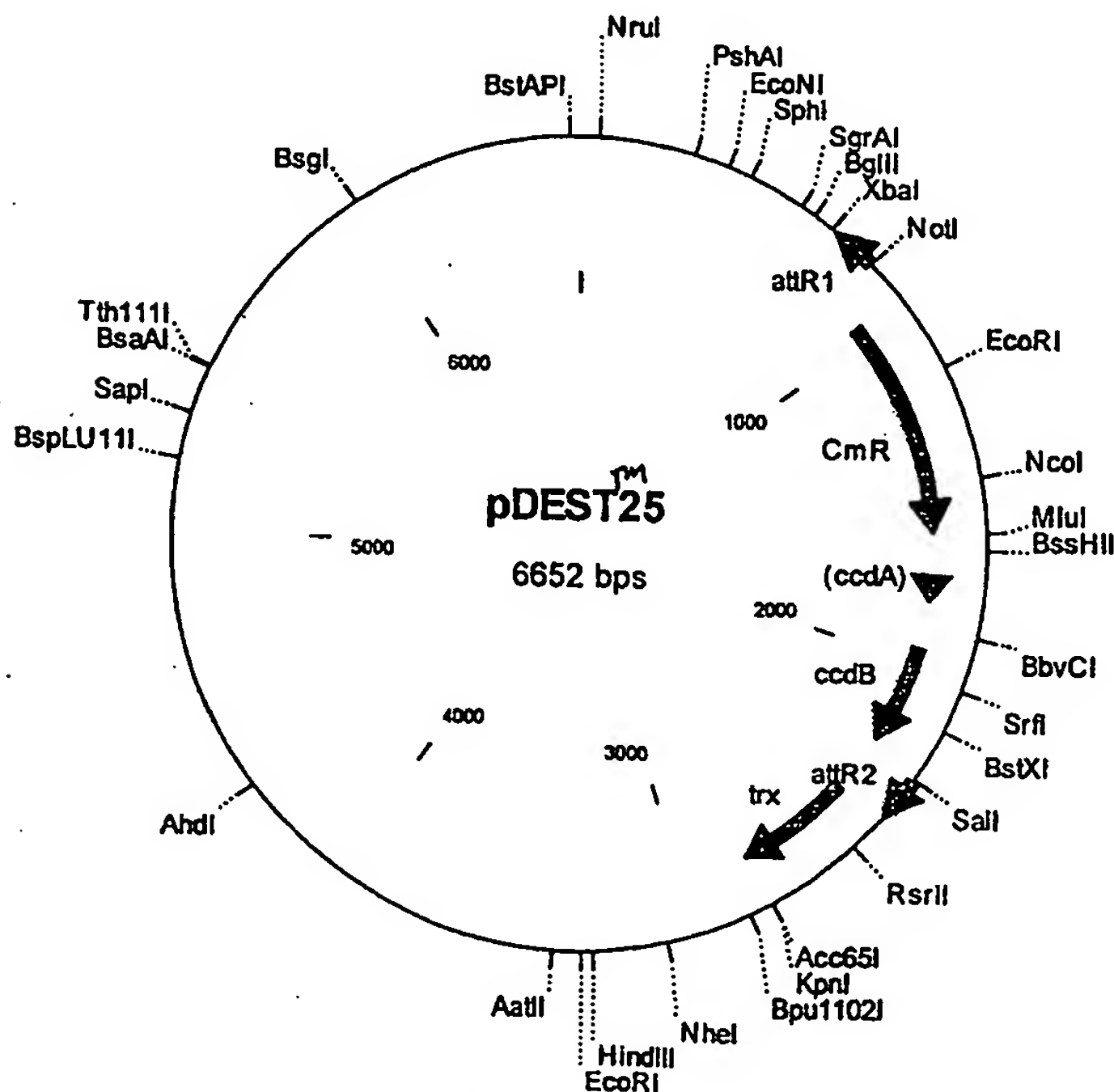
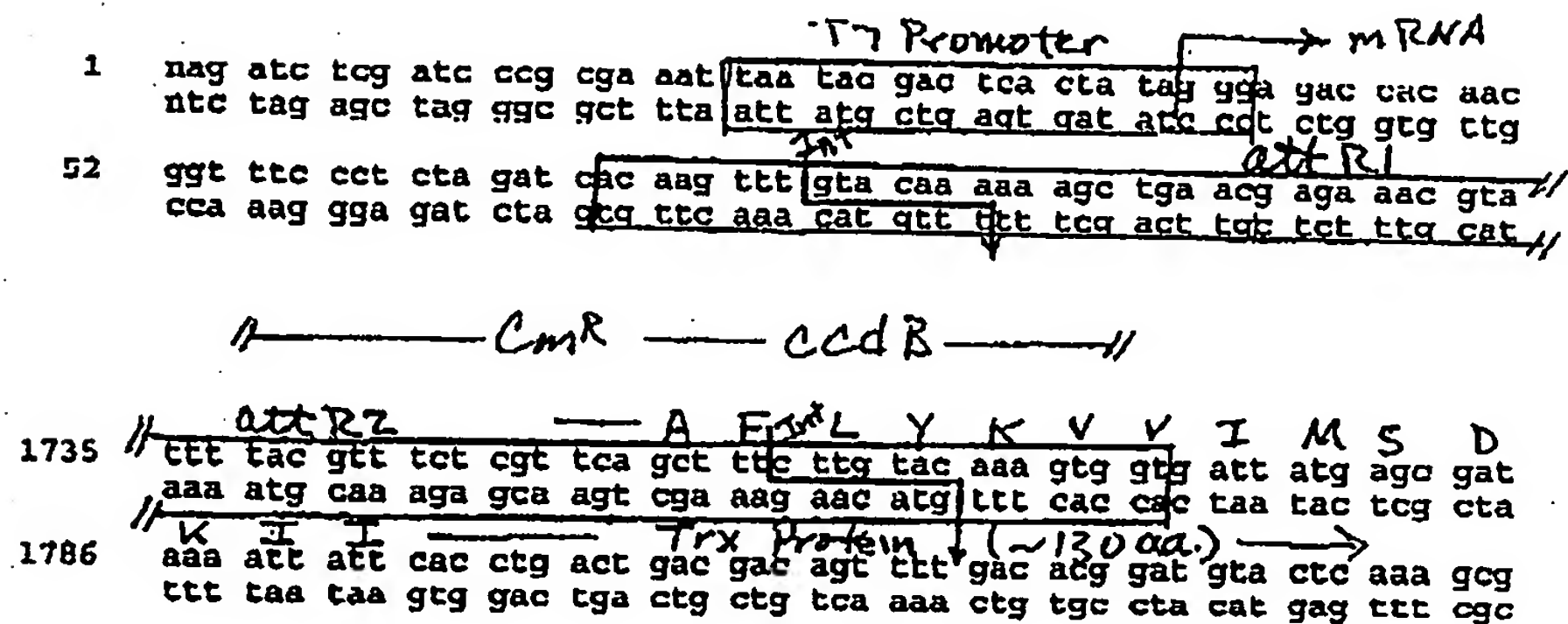


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FIGURE 45A

pDEST25

Thioredoxin carboxy-fusion vector, T7 promoter



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## pDEST25 6652 bp

<u>Location (Base Nos.)</u>		<u>Gene Encoded</u>
844..720		attR1
953..1612		CmR
1732..1816		inactivated ccdA
1954..2259		ccdB
2300..2424		attR2
2432..2794		trx
1	CCGGAAGCGA GAAGAATCAT AATGGGGAAG GCCATCCAGC CTCGCGTCGC GAACGCCAGC	
61	AAGACGTAGC CCAGCGCGTC GGCCGCCATG CCGGCGATAA TGGCCTGCTT CTCGCCGAAA	
121	CGTTTGGTGG CGGGACCAGT GACGAAGGCT TGAGCGAGGG CGTGCAAGAT TCCGAATACC	
181	GCAAGCGACA GGCCGATCAT CGTCGCGCTC CAGCGAAAGC GGTCCCTCGCC GAAAATGACC	
241	CAGAGCGCTG CCGGCACCTG TCCTACGAGT TGCATGATAA AGAAGACAGT CATAAGTGCG	
301	GCGACGATAG TCATGCCCCG CGCCACCCGG AAGGAGCTGA CTGGGTTGAA GGCTCTCAAG	
361	GGCATCGGTC GATCGACGCT CTCCTTATG CACTCCTGC ATTAGGAAGC AGCCAGTAG	
421	TAGGTTGAGG CCGTTGAGCA CCGCCGCCGC AAGGAATGGT GCATGCAAGG AGATGGCGCC	
481	CAACAGTCCC CCGGCCACGG GGCCTGCCAC CATACCCACG CCGAAACAAG CGCTCATGAG	
541	CCCGAAGTGG CGAGCCCGAT CTTCCCCATC GGTGATGTCG GCGATATAGG CGCCAGCAAC	
601	CGCACCTGTG GCGCCGGTGA TGCCGCCAC GATGCGTCCG GCGTAGAGGA TCGAGATCTC	
661	GATCCCGCGA AATTAATACG ACTCACTATA GGGAGACCAC AACGGTTTCC CTCTAGATCA	
721	CAAGTTTGTG CAAAAAAGCT GAACGAGAAA CGTAAATGA TATAAATATC AATATATTAA	
781	ATTAGATTTT GCATAAAAAA CAGACTACAT AATACTGTAA AACACAACAT ATCCAGTCAC	
841	TATGGCGGCC GCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATAATGT	
901	GTGGATTTTG AGTTAGGATC CGGCGAGATT TTCAGGAGCT AAGGAAGCTA AAATGGAGAA	
961	AAAAATCACT GGATATACCA CCGTTGATAT ATCCCAATGG CATCGTAAAG AACATTTTGA	
1021	GGCATTTTCAG TCAGTTGCTC AATGTACCTA TAACCAGACC GTTCAGCTGG ATATTACGGC	
1081	CTTTTAAAG ACCGTAAAGA AAAATAAGCA CAAGTTTTAT CCGGCCTTTA TTCACATTCT	
1141	TGCCCGCCTG ATGAATGCTC ATCCGGAATT CCGTATGGCA ATGAAAGACG GTGAGCTGGT	
1201	GATATGGGAT AGTGTTACAC CTTGTTACAC CGTTTTCCAT GAGCAAACCTG AAACGTTTTTC	
1261	ATCGCTCTGG AGTGAATACC ACGACGATTT CCGGCAGTTT CTACACATAT ATTGCAAGA	
1321	TGTGGCGTGT TACGGTGAAA ACCTGGCCTA TTTCCCTAAA GGGTTTATTG AGAATATGTT	
1381	TTTCGTCTCA GCCAATCCCT GGGTGAGTTT CACCAGTTTT GATTAAACG TGGCCAATAT	
1441	GGACAACTTC TTCGCCCCCG TTTTCACCAT GGGCAAATAT TATACGCAAG GCGACAAGGT	
1501	GCTGATGCCG CTGGCGATTC AGGTTTCATCA TGCCGTCTGT GATGGCTTCC ATGTCGGCAG	
1561	AATGCTTAAT GAATTACAAC AGTACTGCGA TGAGTGCCAG GGCGGGGCGT AAACGCGTGG	
1621	ATCCGGCTTA CTAAAAGCCA GATAACAGTA TGCGTATTTG CGCGCTGATT TTTGCGGTAT	
1681	AAGAATATAT ACTGATATGT ATACCCGAAG TATGTCAAAA AGAGGTGTGC TATGAAGCAG	
1741	CGTATTACAG TGACAGTTGA CAGCGACAGC TATCAGTTGC TCAAGGCATA TATGATGTCA	
1801	ATATCTCCGG TCTGGTAAGC ACAACCATGC AGAATGAAGC CCGTCGTCTG CGTGCCGAAC	
1861	GCTGGAAAGC GGAAAATCAG GAAGGGATGG CTGAGGTCCG CCGGTTTATT GAAATGAACG	
1921	GCTCTTTTGC TGACGAGAAC AGGGACTGGT GAAATGCAGT TTAAGGTTTA CACCTATAAA	
1981	AGAGAGAGCC GTTATCGTCT GTTTGTGGAT GTACAGAGTG ATATTATTGA CACGCCCGGG	
2041	CGACGGATGG TGATCCCCCT GGCCAGTGCA CGTCTGCTGT CAGATAAAGT CTCCCGTGAA	
2101	CTTTACCCGG TGGTGCATAT CGGGGATGAA AGCTGGCGCA TGATGACCAC CGATATGGCC	
2161	AGTGTGCCGG TCTCCGTTAT CGGGGAAGAA GTGGCTGATC TCAGCCACCG CGAAAATGAC	
2221	ATCAAAAACG CCATTAACCT GATGTTCTGG GGAATATAAA TGTCAGGCTC CCTTATACAC	
2281	AGCCAGTCTG CAGGTCGACC ATAGTGACTG GATATGTTGT GTTTTACAGT ATTATGTAGT	
2341	CTGTTTTTTA TGCAAAATCT AATTTAATAT ATTGATATTT ATATCATTTT ACGTTTCTCG	
2401	TTGAGCTTTC TTGTACAAAG TGGTGATTAT GAGCGATAAA ATTATTCACC TGAATGACGA	
2461	CAGTTTTGAC ACGGATGTAC TCAAAGCGGA CGGGGCGATC CTCGTCGATT TCTGGGCAGA	
2521	GTGGTGCGGT CCGTGCAAAA TGATCGCCCC GATTCTGGAT GAAATCGCTG ACGAATATCA	
2581	GGGCAAACTG ACCGTTGCAA AACTGAACAT CGATCAAAAC CCTGGCACTG CGCCGAAATA	
2641	TGGCATCCGT GGTATCCCGA CTCTGCTGCT GTTCAAAAAC GGTGAAGTGG CGGCAACCAA	
2701	AGTGGGTGCA CTGTCTAAAG GTCAGTTGAA AGAGTTCCTC GACGCTAACC TGGCCGGTTC	
2761	TGGTTCTGGT GATGACGATG ACAAGGTACC CGGGGATCGA TCCGGCTGCT AACAAAGCCC	

Figure 45B

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2821 GAAAGGAAGC TGAGTTGGCT GCTGCCACCG CTGAGCAATA ACTAGCATAA CCCCTTGGGG  
2881 CCTCTAAACG GGTCTTGAGG GGTTTTTTGTC TGAAAGGAGG AACTATATCC GGATATCCAC  
2941 AGGACGGGTG TGGTCGCCAT GATCGCGTAG TCGATAGTGG CTCCAAGTAG CGAAGCGAGC  
3001 AGGACTGGGC GGCGGCCAAA GCGGTCGGAC AGTGCTCCGA GAACGGGTGC GCATAGAAAT  
3061 TGCATCAACG CATATAGCGC TAGCAGCACG CCATAGTGAC TGGCGATGCT GTCGGAATGG  
3121 ACGATATCCC GCAAGAGGCC CGGCAGTACC GGCATAACCA AGCCTATGCC TACAGCATCC  
3181 AGGGTGACGG TGCCGAGGAT GACGATGAGC GCATTGTTAG ATTTTCATACA CCGTGCCCTGA  
3241 CTGCGTTAGC AATTTAAC TGATAAACTA CCGCATTAAA GCTTATCGAT GATAAGCTGT  
3301 CAAACATGAG AATTCTTGAA GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA  
3361 TGTCATGATA ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG  
3421 AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA  
3481 ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG  
3541 TGTCGCCCTT ATTCCCTTTT TTGCGGCATT TTGCCCTTCT GTTTTTGCTC ACCCAGAAAC  
3601 GCTGGTGAAA GTAAAAGATG CTGAAGATCA GTTGGGTGCA CGAGTGGGT ACATCGAACT  
3661 GGATCTCAAC AGCGGTAAGA TCCTTGAGAG TTTCGCCCC GAAGAACGTT TTCCAATGAT  
3721 GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTGTGACG CCGGGCAAGA  
3781 GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC  
3841 AGAAAAGCAT CTTACGGATG GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT  
3901 GAGTGATAAC ACTGCGGCCA ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC  
3961 CGCTTTTTTG CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCAGGAGCT  
4021 GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGCAGCAA TGGCAACAAC  
4081 GTTGCAGCAA CTATTAAC TGCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA  
4141 CTGGATGGAG GCGGATAAAG TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG  
4201 GTTTATTGCT GATAAATCTG GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT  
4261 GGGGCCAGAT GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC  
4321 TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA  
4381 ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT TTAAAACCTC ATTTTTAATT  
4441 TAAAAGGATC TAGGTGAAGA TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA  
4501 GTTTTCGTTT CACTGAGCGT CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC  
4561 TTTTTTCTG CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT  
4621 TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC  
4681 GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC  
4741 TGAGCACCG CCTACATACC TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG  
4801 CGATAAGTCG TGTCTTACCG GGTGAGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG  
4861 GTCGGGCTGA ACGGGGGGTT CGTGACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA  
4921 ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC  
4981 GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG  
5041 GGGAAACGCC TGGTATCTTT ATAGTCCTGT CCGGTTTTGCG CACCTCTGAC TTGAGCGTCG  
5101 ATTTTTGTGA TGCTCGTCAG GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT  
5161 TTTACGGTTC CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC  
5221 TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG  
5281 AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA GAGCGCCTGA TCGGGTATTT  
5341 TCTCCTTACG CATCTGTGCG GTATTTTACA CCGCATATAT GGTGCACTCT CAGTACAATC  
5401 TGCTCTGATG CCGCATAGTT AAGCCAGTAT ACACTCCGCT ATCGCTACGT GACTGGGTCA  
5461 TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC  
5521 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT  
5581 CACCGTCATC ACCGAAACGC GCGAGGCAGC TGCGGTAAAG CTCATCAGCG TGGTCGTGAA  
5641 GCGATTACCA GATGTCTGCC GTTTCATCCG CGTCCAGCTC GTTGAGTTTC TCCAGAAGCG  
5701 TTAATGTCTG GCTTCTGATA AAGCGGGCCA TGTTAAGGGC GGTTTTTTCC TGTTTGGTCA  
5761 CTGATGCCTC CGTGTAAAGG GGATTTCTGT TCATGGGGGT AATGATACCG ATGAAACGAG  
5821 AGAGGATGCT CACGATACGG GTTACTGATG ATGAACATGC CCGGTTACTG GAACGTTGTG  
5881 AGGGTAAACA ACTGGCGGTA TGGATGCGGC GGGACCAGAG AAAAATCACT CAGGGTCAAT  
5941 GCCAGCGCTT CGTTAATACA GATGTAGGTG TTCCACAGGG TAGCCAGCAG CATCCTGCGA  
6001 TGCAGATCCG GAACATAATG GTGCAGGGCG CTGACTTCCG CGTTTCCAGA CTTTACGAAA  
6061 CACGGAAACC GAAGACCATT CATGTTGTTG CTCAGGTCGC AGACGTTTTG CAGCAGCAGT  
6121 CGCTTCACGT TCGCTCGCGT ATCGGTGATT CATTCTGCTA ACCAGTAAGG CAACCCCGCC  
6181 AGCCTAGCCG GGTCTCAAC GACAGGAGCA CGATCATGCG CACCCGTGGC CAGGACCCAA  
6241 CGCTGCCCGA GATGCGCCGC GTGCGGCTGC TGGAGATGGC GGACGCGATG GATATGTTCT

FIGURE 45C

6301 GCCAAGGGTT GGTTCGCA TTCACAGTTC TCCGCAAGAA TTGATTGGCT CCAATTCTTG  
6361 GAGTGGTGAA TCCGTTAGCG AGGTGCCGCC GGCTTCCATT CAGGTCGAGG TGGCCCGGCT  
6421 CCATGCACCG CGACGCAACG CGGGGAGGCA GACAAGGTAT AGGGCGGCGC CTACAATCCA  
6481 TGCCAACCCG TTCCATGTGC TCGCCGAGGC GGCATAAATC GCCGTGACGA TCAGCGGTCC  
6541 AGTGATCGAA GTTAGGCTGG TAAGAGCCGC GAGCGATCCT TGAAGCTGTC CCTGATGGTC  
6601 GTCATCTACC TGCCTGGACA GCATGGCCTG CAACGCGGGC ATCCCGATGC CG

## FIGURE 46A

## pDEST26 His6 Amino Fusion in pCMV Sport-neo Vector

600 ttg acg tca atg gga gtt tgt ttt ggc acc aaa atc aac ggg act ttc caa  
aac tgc agt tac cct caa aca aaa ccg tgg ttt tag ttg ccc tga aag gtt

651 aat gtc gta aca act ccg ccc oat tga cgc aaa tgg gcg gta ggc gtg tac  
tta cag cat tgt tga ggc ggg gta act ggc ttt acc cgc cat ccg cac atg

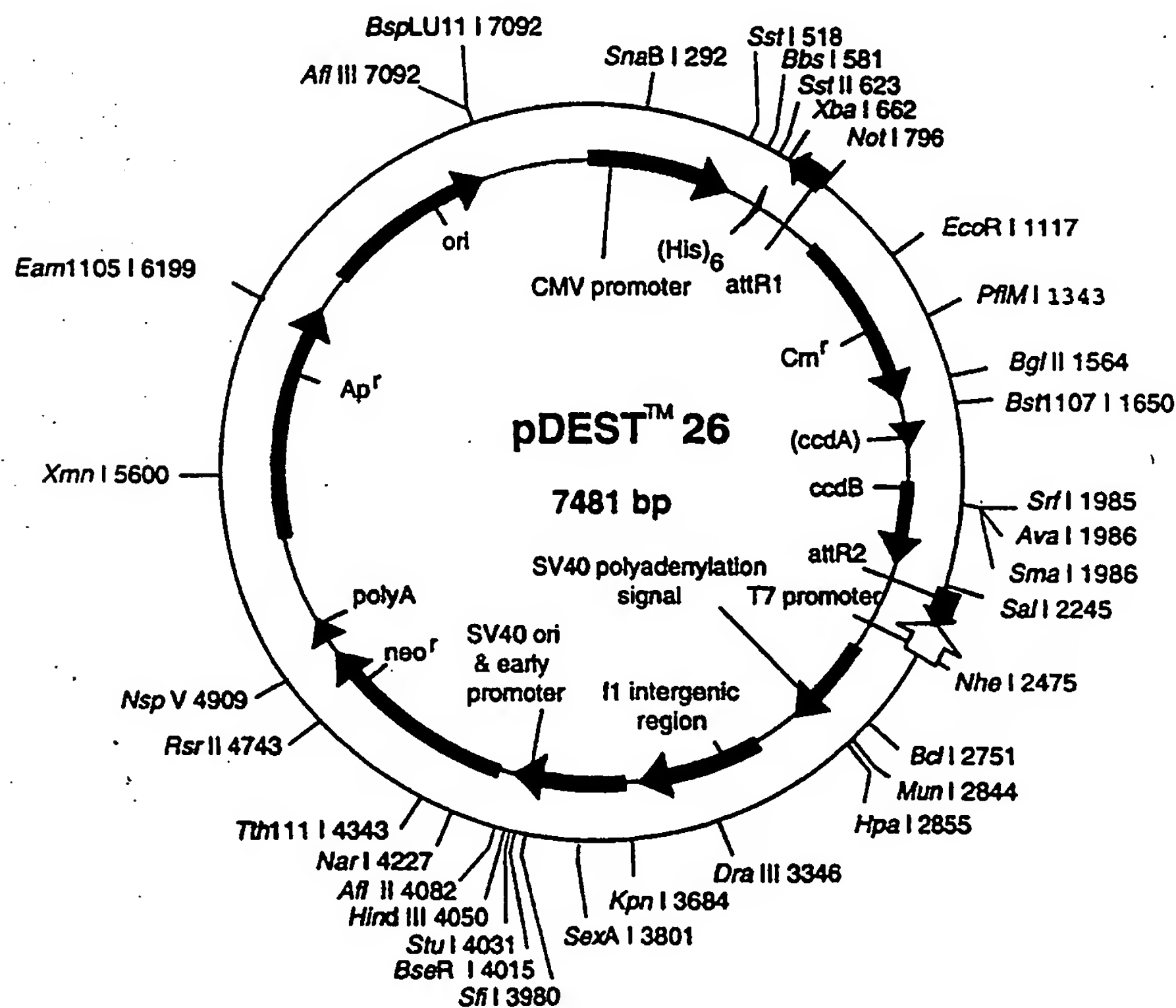
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//cca ccc tcc aga tat att cgt ctc gag caa atc act tgg cag tct agc gga

753 gga gac gcc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc gat  
cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg cta

804 cca gcc tcc gga etc tag cct agg ccg cgg acc atg gcg tac tac cat cac  
ggc cgg agg cct gag atc gga tcc ggc gcc tgg tac cgc atg atg gta gta

855 H H H H S R S I S I V K K A  
cat cac cat cac tct aga tca aca agt ttg tac aaa aaa gct gaa cga gaa  
gta gtg gta gtg aga tct agt tgt tca aac atg ttt ttt cga att gct ctt

CMV Promoter  
Start Transl  
Int Y





## pDEST26 7481 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
492..509	his6
619..519	attR1
752..1411	CmR
1531..1615	inactivated ccdA
1753..2058	ccdB
2099..2223	attR2
2409..2771	SV40 polyA
2966..3421	f1 intergenic region
3485..3903	SV40 promoter
3948..4742	neo
4806..4854	polyA
5265..6125	Apr
6274..6913	ori
7344..385	CMV promoter

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61 CGTCAATGAC GGTAATGGC CCGCCTGGCA TTATGCCAGC TACATGACCT TATGGGACTT
121 TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT ACCATGGTGA TGCGGTTTGT
181 GCAGTACATC AATGGGCGTG GATAGCGGTT TGA CTACCGG GGATTTCCAA GTCTCCACCC
241 CATTGACGTC AATGGGAGTT TGT TTTGGCA CCAAATCAA CGGGACTTTC CAAATGTGCG
301 TAACAACTCC GCCCATTTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
361 AAGCAGAGCT CGTTTAGTGA ACCGTCAGAT CGCCTGGAGA CGCCATCCAC GCTGTTTGA
421 CCTCCATAGA AGACACCGGG ACCGATCCAG CCTCCGGACT CTAGCCTAGG CCGCGGACCA
481 TGGCGTACTA CCATCACCAT CACCATCACT CTAGATCAAC AAGTTTGTAC AAAAAAGCTG
541 AACGAGAAAC GTAAATGAT ATAAATATCA ATATATTAAA TTAGATTTTG CATAAAAAAC
601 AGACTACATA ATACTGTAAA ACACAACATA TCCAGTCACT ATGGCGGCCG CATTAGGCAC
661 CCCAGGCTTT ACAC TTTATG CTTCCGGCTC GTATAATGTG TGGATTTTGA GTTAGGATCC
721 GGCAGATTTT TCAGGAGCTA AGGAAGCTAA AATGGAGAAA AAAATCACTG GATATACCAC
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901 AAATAAGCAC AAGTTTATC CGGCCTTTAT TCACATTCTT GCCCGCCTGA TGAATGCTCA
961 TCCGGAATTC CGTATGGCAA TGAAAGACGG TGAGCTGGTG ATATGGGATA GTGTTACACC
1021 TTGTTACACC GTTTTCCATG AGCAAACTGA AACGTTTTCA TCGCTCTGGA GTGAATACCA
1081 CGACGATTTT CGGCAGTTTC TACACATATA TTCGCAAGAT GTGGCGTGTT ACGGTGAAAA
1141 CCTGGCCTAT TTCCCTAAAG GGT TATTGA GAATATGTTT TTCGTCTCAG CCAATCCCTG
1201 GGTGAGTTTC ACCAGTTTGT ATTTAAACGT GGCCAATATG GACAACCTCT TCGCCCCCGT
1261 TTTACCATG GCAAAATATT ATACGCAAGG CGACAAGGTG CTGATGCCGC TGGCGATTCA
1321 GGTTCATCAT GCCGTCTGTG ATGGCTTCCA TGTCGGCAGA ATGCTTAATG AATTACAACA
1381 GTACTGCGAT GAGTGGCAGG GCGGGGCGTA AAGATCTGGA TCCGGCTTAC TAAAGCCAG
1441 ATAACAGTAT GCGTATTTGC GCGCTGATTT TTGCGGTATA AGAATATATA CTGATATGTA
1501 TACCCGAAGT ATGTCAAAAA GAGGTGTGCT ATGAAGCAGC GTATTACAGT GACAGTTGAC
1561 AGCGACAGCT ATCAGTTGCT CAAGGCATAT ATGATGTCAA TATCTCCGGT CTGGTAAGCA
1621 CAACCATGCA GAATGAAGCC CGTCGTCTGC GTGCCGAACG CTGGAAAGCG GAAATCAGG
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1861 GCCAGTGCAC GTCTGCTGTC AGATAAAGTC TCCCGTGAAC TTTACCCGGT GGTGCATATC
1921 GGGGATGAAA GCTGGCGCAT GATGACCACC GATATGGCCA GTGTGCCGGT CTCCGTTATC
1981 GGGGAAGAAG TGGCTGATCT CAGCCACCGC GAAAATGACA TCAAAAACGC CATTAAACCTG
2041 ATGTTCTGGG GAATATAAAT GTCAGGCTCC CTTATACACA GCCAGTCTGC AGGTGACCA
2101 TAGTGA CTGG ATATGTTGTG TTTTACAGTA TTATGTAGTC TGTTTTTTAT GCAAAATCTA
2161 ATTTAATATA TTGATATTTA TATCATTTTA CGTTTCTCGT TCAGCTTTCT TGTACAAAGT
2221 GGTGATCGC GTGCATGCGA CGTCATAGCT CTCTCCCTAT AGTGAGTCGT ATTATAAGCT
2281 AGGCACTGGC CGTCGTTTTA CAACGTCGTG ACTGGGAAAA CTGCTAGCTT GGGATCTTTG -

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2341 TGAAGGAACC TTACTTCTGT GGTGTGACAT AATTGGACAA ACTACCTACA GAGATTTAAA  
2401 GCTCTAAGGT AAATATAAAA TTTTAAAGTG TATAATGTGT TAAACTAGCT GCATATGCTT  
2461 GCTGCTTGAG AGTTTGTCTT ACTGAGTATG ATTTATGAAA ATATTATACA CAGGAGCTAG  
2521 TGATTCTAAT TGTGTGTGTA TTTTAGATTG ACAGTCCCAA GGCTCATTTC AGGCCCTCA  
2581 GTCCTCACAG TCTGTTTCATG ATCATAATCA GCCATACCAC ATTTGTAGAG GTTTTACTTG  
2641 CTTTAAAAAA CCTCCCACAC CTCCCCCTGA ACCTGAAACA TAAATGAAT GCAATTGTTG  
2701 TTGTTAACTT GTTTATTGCA GCTTATAATG GTTACAAATA AAGCAATAGC ATCACAAATT  
2761 TCACAAATAA AGCATTTTTTT TCACTGCATT CTAGTTGTGG TTTGTCCAAA CTCATCAATG  
2821 TATCTTATCA TGTCTGGATC GATCCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC  
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2941 TTGCGCAGCC TGAATGGCGA ATGGGACGCG CCCTGTAGCG GCGCATTAAAG CGCGGCGGGT  
3001 GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC  
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3121 GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT  
3181 TAGGGTGATG GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG  
3241 TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC ACTCAACCCT  
3301 ATCTCGGTCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCCGCCCTA TTGGTTAAAA  
3361 AATGAGCTGA TTTAACAAT ATTTAACGCG AATTTTAAAC AAATATTAAC GTTTACAATT  
3421 TCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTACACACC GCATACGCGG  
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3781 TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC  
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3901 CTTGATTCTT CTGACACAAC AGTCTCGAAC TTAAGGCTAG AGCCACCATG ATTGAACAAG  
3961 ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTTCGC TATGACTGGG  
4021 CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCG GCTGTCAGCG CAGGGGCGCC  
4081 CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAAGTGCAG GACGAGGCAG  
4141 CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC GACGTTGTCA  
4201 CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT CTCCTGTCTAT  
4261 CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG CGGCTGCATA  
4321 CGCTTGATCC GGCTACCTGC CCATTGACG ACCAAGCGAA ACATCGCATC GAGCGAGCAC  
4381 GTAATCGGAT GGAAGCCGGT CTTGTGATC AGGATGATCT GGACGAAGAG CATCAGGGGC  
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4561 GATTCATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA  
4621 CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCCTC GTGCTTTACG  
4681 GTATCGCCGC TCCCGATTCC CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTTCCTCT  
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4801 GCCGCAATAA AATATCTTTA TTTTCATTAC ATCTGTGTGT TGGTTTTTTG TGTGAATCGA  
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4981 CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT  
5041 CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGCCTCGT GATACGCCTA TTTTATAGG  
5101 TTAATGTCAT GATAATAATG GTTCTTTAGA CGTCAGGTGG CACTTTTCGG GGAAATGTGC  
5161 GCGGAACCCC TATTTGTTTA TTTTCTTAAA TACATTCAA TATGTATCCG CTCATGAGAC  
5221 AATAACCCTG ATAAATGCTT CAATAATATT GAAAAAGGAA GAGTATGAGT ATCAACATT  
5281 TCCGTGTGCG CCTTATTCCC TTTTGTGCGG CATTTTGCCT TCCTGTTTTT GCTCACCAG  
5341 AAACGCTGGT GAAAGTAAAA GATGCTGAAG ATCAGTTGGG TGCACGAGTG GGTACATCG  
5401 AACTGGATCT CAACAGCGGT AAGATCCTTG AGAGTTTTTC CCCCAGAGAA CGTTTTTCAA  
5461 TGATGAGCAC TTTTAAAGTT CTGCTATGTG GCGCGGTATT ATCCCGTATT GACGCCGGGC  
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5821 CAACGTTGCG CAAACTATTA ACTGGCGAAC TACTTACTCT AGCTTCCCGG CAACAATTAA  
5881 TAGACTGGAT GGAGGCGGAT AAAGTTGCAG GACCACTTCT GCGCTCGGCC CTTCCGGCTG  
5941 GCTGGTTTAT TGCTGATAAA TCTGGAGCCG GTGAGCGTGG GTCTCGCGGT ATCATTGCAG  
6001 CACTGGGGCC AGATGGTAAG CCCTCCCGTA TCGTAGTTAT CTACACGACG GGGAGTCAGG  
6061 CAACTATGGA TGAACGAAAT AGACAGATCG CTGAGATAGG TGCCTCACTG ATTAAGCATT  
6121 GGTAAC TGTC AGACCAAGTT TACTCATATA TACTTTAGAT TGATTTAAAA CTTCAATTTT  
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6301 ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG CTACCAGCGG  
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6421 GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC CACTTCAAGA  
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6661 CCGAAGTGAG ATACCTACAG CGTGAGCATT GAGAAAGCGC CACGCTTCCC GAAGGGAGAA  
6721 AGGCGGACAG GTATCCGGTA AGCGGCAGGG TCGGAACAGG AGAGCGCACG AGGGAGCTTC  
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7441 ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC G

136/240  
FIGURE 47A

# pDEST 27 GST Amino Fusion in pCMV Sport-neo Vector

CMV Promoter

600 // nac ggt ggg agg tct ata taa gca gag ctc gtc tag tga acc gtc aya tcy  
ntg cca ccc tcc aga tat att cgt ctc gag caa atc act tgg dag tct agc

651 cct gga gac gcc atc cac gct gtt ttg acc tcc ata gaa gac acc ggg acc  
gga cct ctg cgg tag gtg cga caa aac tgg agg tat ctt ctg tgg ccc tgg

702 gat cca gcc tcc gga ctc tag cct agg cgg cgg acc atg gcc cct ata cta  
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Start Transl GST

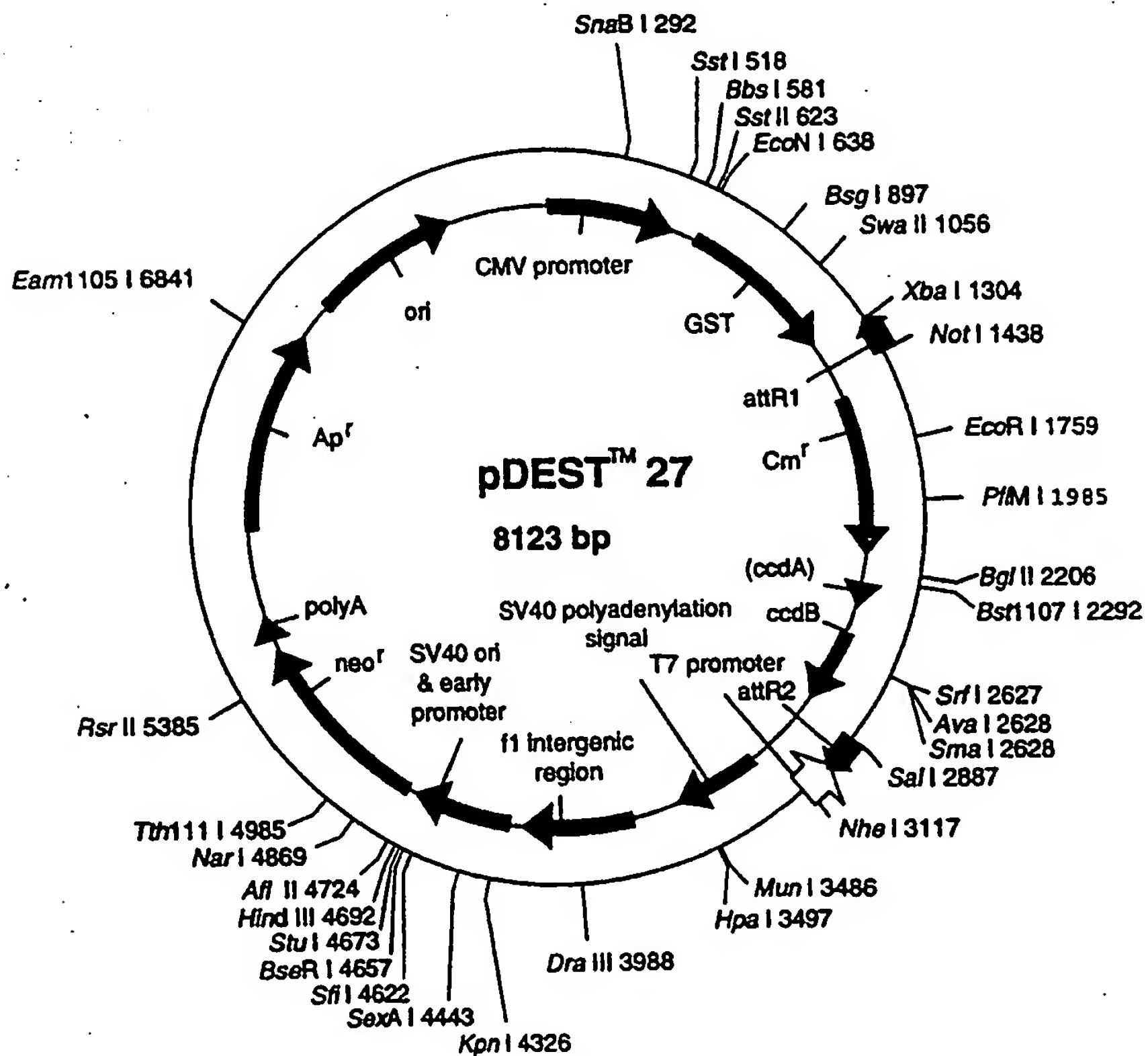
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cca ata acc ttt taa ttc ccg gaa cac gtt ggg tga gct gaa gaa aac ctt

804 tat ctt gaa gaa aaa tat gaa gag cat ttg tat gag cgc gat gaa ggt gat  
ata gaa ctt ctt ttt ata ctt ctc gta aac ata ctc gcg cta ctt cca cta

1365 // ttt ggt ggt ggc gac cat cct cca aaa tcy gat ctg gtt ccg cgt tct aga  
aaa cca cca ccg ctg gta gga ggt ttt agc cta gac caa ggc gca aga tct

1416 tca aca agt ttg tac aaa aaa gct gaa cga gaa acg  
agt tgt tca aac atg ttt ttt cga ctt gct ctt tgc

Int attR1



## pDEST27 8123 bp (rotated to position 7800)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
130..793	GST
803..927	attR1
1036..1695	CmR
1815..1899	inactivated ccdA
2037..2342	ccdB
2383..2507	attR2
2693..3055	SV40 polyA
3250..3705	f1 intergenic region
3769..4187	SV40 promoter
4232..5026	neo
5090..5138	polyA
5549..6409	Apr
6558..7197	ori
7628..27	CMV promoter

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61 GACCTCCATA GAAGACACCG GGACCGATCC AGCCTCCGGA CTCTAGCCTA GGCCGCGGAC
121 CATGGCCCCT ATACTAGGTT ATTGGAAAAT TAAGGGCCTT GTGCAACCCA CTCGACTTCT
181 TTTGGAATAT CTTGAAGAAA AATATGAAGA GCATTTGTAT GAGCGCGATG AAGGTGATAA
241 ATGGCGAAAC AAAAAGTTTG AATTGGGTTT GGAGTTTCCC AATCTTCCTT ATTATATTGA
301 TGGTGATGTT AAATTAACAC AGTCTATGGC CATCATACGT TATATAGCTG ACAAGCACAA
361 CATGTTGGGT GGTGTGCCAA AAGAGCGTGC AGAGATTTC A TGCTTGAAG GAGCGGTTTT
421 GGATATTAGA TACGGTGTTC CGAGAATTGC ATATAGTAAA GACTTTGAAA CTCTCAAAGT
481 TGATTTTCTT AGCAAGCTAC CTGAAATGCT GAAAATGTTC GAAGATCGTT TATGTCATAA
541 AACATATTTA AATGGTGATC ATGTAACCCA TCCTGACTTC ATGTTGTATG ACGCTCTTGA
601 TGTGTGTTTA TACATGGACC CAATGTGCCT GGATGCGTTC CCAAATTAG TTTGTTTTAA
661 AAAACGTATT GAAGCTATCC CACAAATTGA TAAGTACTTG AAATCCAGCA AGTATATAGC
721 ATGGCCTTTG CAGGGCTGGC AAGCCACGTT TGGTGGTGGC GACCATCCTC CAAAATCGGA
781 TCTGGTTCCG CGTTCTAGAT CAACAAGTTT GTACAAAAAA GCTGAACGAG AAACGTAAAA
841 TGATATAAAT ATCAATATAT TAAATTAGAT TTTGCATAAA AAACAGACTA CATAATACTG
901 TAAAACACAA CATATCCAGT CACTATGGCG GCCGCATTAG GCACCCCGAG CTTTACACTT
961 TATGCTTCCG GCTCGTATAA TGTGTGGATT TTGAGTTAGG ATCCGGCGAG ATTTTCAGGA
1021 GCTAAGGAAG CTAAAATGGA GAAAAAATC ACTGGATATA CCACCGTTGA TATATCCCAA
1081 TGGCATCGTA AAGAACATTT TGAGGCATTT CAGTCAGTTG CTCAATGTAC CTATAACCAG
1141 ACCGTTTACG TGGATATTAC GGCCTTTTTA AAGACCGTAA AGAAAAATAA GCACAAGTTT
1201 TATCCGGCCT TTATTCACAT TCTTGCCCGC CTGATGAATG CTCATCCGGA ATTCGGTATG
1261 GCAATGAAAG ACGGTGAGCT GGTGATATGG GATAGTGTTT ACCCTTGTTA CACCGTTTTT
1321 CATGAGCAAA CTGAAACGTT TTCATCGCTC TGGAGTGAAT ACCACGACGA TTTCCGGCAG
1381 TTTCTACACA TATATTCGCA AGATGTGGCG TGTACGGTG AAAACCTGGC CTATTTCCCT
1441 AAAGGGTTTA TTGAGAATAT GTTTTTCGTC TCAGCCAATC CCTGGGTGAG TTTCACCAGT
1501 TTTGATTTAA ACGTGGCCAA TATGGACAAC TTCTTCGCCC CCGTTTTTCAC CATGGGCAAA
1561 TATTATACGC AAGGCGACAA GGTGCTGATG CCGCTGGCGA TTCAGGTTCA TCATGCCGTC
1621 TGTGATGGCT TCCATGTCGG CAGAATGCTT AATGAATTAC AACAGTACTG CGATGAGTGG
1681 CAGGGCGGGG CGTAAAGATC TGGATCCGGC TTAATAAAAG CCAGATAACA GTATGCGTAT
1741 TTGCGCGCTG ATTTTTCGCG TATAAGAATA TATACTGATA TGTATACCCG AAGTATGTCA
1801 AAAAGAGGTG TGCTATGAAG CAGCGTATTA CAGTGACAGT TGACAGCGAC AGCTATCAGT
1861 TGCTCAAGGC ATATATGATG TCAATATCTC CGGTCTGGTA AGCACAACCA TGCAGAATGA
1921 AGCCCGTCGT CTGCGTGCCG AACGCTGGAA AGCGGAAAT CAGGAAGGGA TGGCTGAGGT
1981 CGCCCGGTTT ATTGAAATGA ACGGCTCTTT TGCTGACGAG AACAGGGACT GGTGAAATGC
2041 AGTTTAAGGT TTACACCTAT AAAAGAGAGA GCCGTTATCG TCTGTTTGTG GATGTACAGA
2101 GTGATATTAT TGACACGCCG GGGCGACGGA TGGTGATCCC CCTGGCCAGT GCACGTCTGC
2161 TGTCAGATAA AGTCTCCCGT GAACCTTACC CGGTGGTGCA TATCGGGGAT GAAAGCTGGC
2221 GCATGATGAC CACCGATATG GCCAGTGTGC CGGTCTCCGT TATCGGGGAA GAAGTGGCTG
2281 ATCTCAGCCA CCGCGAAAT GACATCAAAA ACGCCATTAA CCTGATGTTT TGGGGAATAT -

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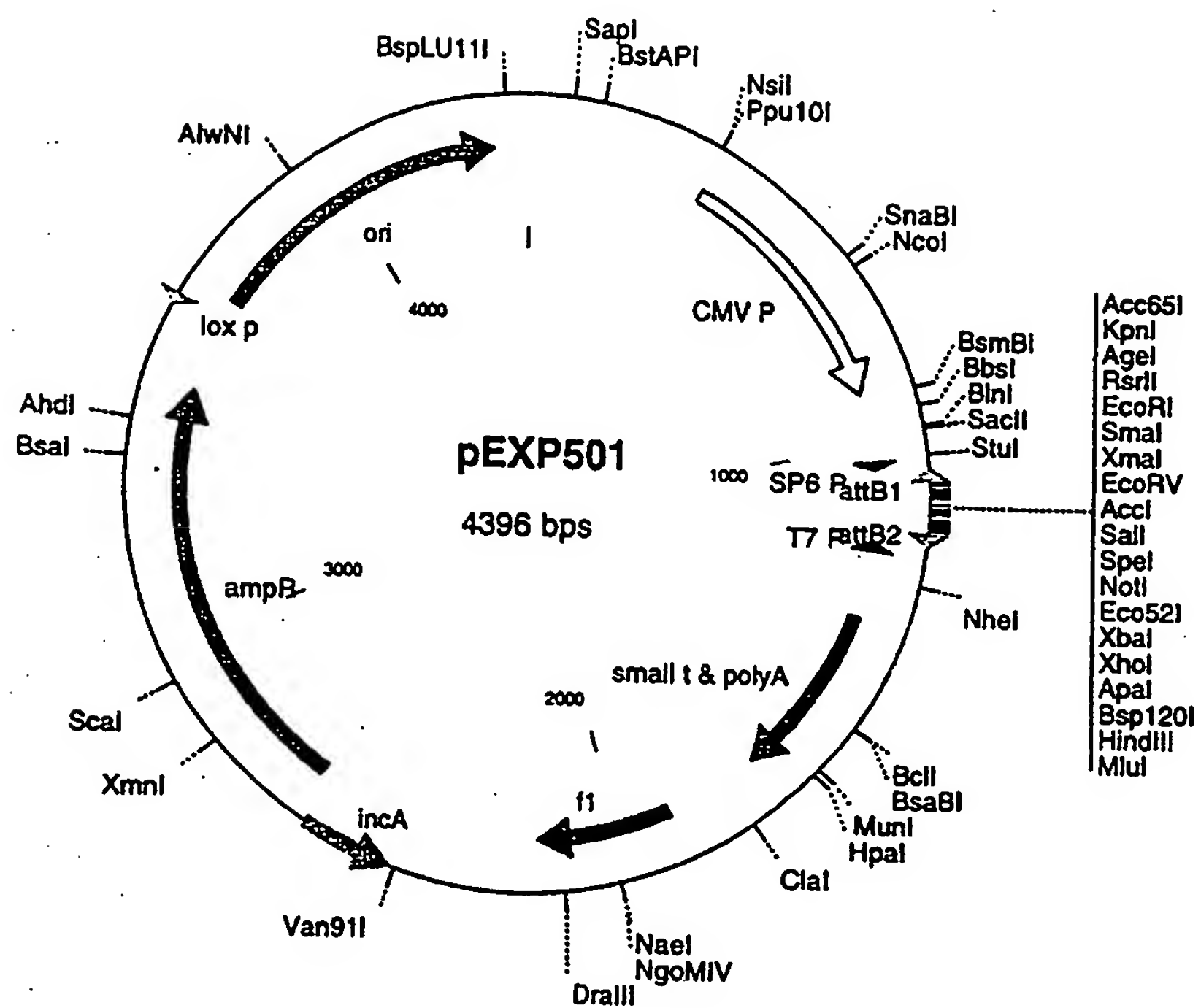


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2521 GCGACGTCAT AGCTCTCTCC CTATAGTGAG TCGTATTATA AGCTAGGCAC TGGCCGTCGT  
2581 TTTACAACGT CGTGACTGGG AAAACTGCTA GCTTGGGATC TTTGTGAAGG AACCTTACTT  
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3001 TGCAGCTTAT AATGGTTACA AATAAGCAA TAGCATCACA AATTTACAA ATAAAGCATT  
3061 TTTTTCACGT CATTCTAGTT GTGGTTTGTG CAAACTCATC AATGTATCTT ATCATGTCTG  
3121 GATCGATCCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGCT  
3181 GCGTAATAG CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGC AGCCTGAATG  
3241 GCGAATGGGA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA  
3301 GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT  
3361 TTCTCGCCAC GTTCGCCGGC TTTCCCGTCC AAGCTCTAAA TCGGGGGGCTC CCTTTAGGGT  
3421 TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTAGGGT GATGGTTCAC  
3481 GTAGTGGGCC ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT  
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3661 AAATATTTAA CGCGAATTTT AACAAAATAT TAACGTTTAC AATTTCCGCT GATGCGGTAT  
3721 TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAC GCGGATCTGC GCAGCACCAT  
3781 GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC  
3841 AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA  
3901 GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC  
3961 CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC  
4021 TAACTCCGCC CATCCCGCCC CTAACCTCCG CCAGTTCCGC CCATTCTCCG CCCCATGGCT  
4081 GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCCTC GGCCTCTGAG CTATTCCAGA  
4141 AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTTGAT TCTTCTGACA  
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4261 TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC AGACAATCGG  
4321 CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTTT TTTTGTCAA  
4381 GACCGACCTG TCCGGTGCCC TGAATGAACT GCAGGACGAG GCAGCGCGGC TATCGTGGCT  
4441 GGCCACGACG GCGGTTCTTT GCGCAGCTGT GCTCGACGTT GTCACTGAAG CGGGAAGGGA  
4501 CTGGCTGCTA TTGGGCGAAG TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC  
4561 CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACGCTTG ATCCGGCTAC  
4621 CTGCCCATTG GACCACCAAG CGAAACATCG CATCGAGCGA GCACGTAATC GGATGGAAGC  
4681 CGGTCTTGTC GATCAGGATG ATCTGGACGA AGAGCATCAG GGGCTCGCGC CAGCCGAATC  
4741 GTTCGCCAGG CTCAAGGCGC GCATGCCCCA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA  
4801 TGCCTGCTTG CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG  
4861 CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA  
4921 AGAGCTTGGC GCGAATGGG CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA  
4981 TTCGACGCGC ATCGCCTTCT ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG  
5041 TTCGAAATGA CCGACCAAGC GACGCCCCAAC CTGCCATCAC GATGGCCGCA ATAAAATATC  
5101 TTTATTTTCA TTACATCTGT GTGTTGGTTT TTTGTGTGAA TCGATAGCGA TAAGGATCCG  
5161 CGTATGGTGC ACTCTCAGTA CAATCTGCTC TGATGCCGCA TAGTTAAGCC AGCCCCGACA  
5221 CCCGCCAACA CCCGCTGACG CGCCCTGACG GGCTTGTCTG CTCCCGGCAT CCGCTTACAG  
5281 ACAAGCTGTG ACCGTCTCCG GGAGCTGCAT GTGTGAGAGG TTTTCACCGT CATCACCAGG  
5341 ACGCGCGAGA CGAAAGGGCC TCGTGATACG CCTATTTTTA TAGGTAAATG TCATGATAAT  
5401 AATGGTTTCT TAGACGTCAG GTGGCACTTT TCGGGGAAAT GTGCGCGGAA CCCCTATTTG  
5461 TTTATTTTTC TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC CCTGATAAAT  
5521 GCTTCAATAA TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT  
5581 TCCCTTTTTT GCGGCATTTT GCCTTCCTGT TTTTGCTCAC CCAGAAACGC TGGTGAAAGT  
5641 AAAAGATGCT GAAGATCAGT TGGGTGCACG AGTGGGTTAC ATCGAACTGG ATCTCAACAG  
5701 CGGTAAGATC CTTGAGAGTT TTCGCCCCGA AGAACGTTTT CCAATGATGA GCACTTTTAA  
5761 AGTTCCTGCTA TGTGGCGCGG TATTATCCCG TATTGACGCC GGGCAAGAGC AACTCGGTCTG

5821 CCGCATACAC TATTCTCAGA ATGACTTGGT TGAGTACTCA CCAGTCACAG AAAAGCATCT  
5881 TACGGATGGC ATGACAGTAA GAGAATTATG CAGTGCTGCC ATAACCATGA GTGATAACAC  
5941 TGCGGCCAAC TTACTTCTGA CAACGATCGG AGGACCGAAG GAGCTAACCG CTTTTTTGCA  
6001 CAACATGGGG GATCATGTAA CTCGCCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT  
6061 ACCAAACGAC GAGCGTGACA CCACGATGCC TGTAGCAATG GCAACAACGT TGCGCAAACT  
6121 ATTAACCTGGC GAACTACTTA CTCTAGCTTC CCGGCAACAA TTAATAGACT GGATGGAGGC  
6181 GGATAAAGTT GCAGGACCAC TTCTGCGCTC GGCCCTTCCG GCTGGCTGGT TTATTGCTGA  
6241 TAAATCTGGA GCCGGTGAGC GTGGGTCTCG CCGTATCATT GCAGCACTGG GGCCAGATGG  
6301 TAAGCCCTCC CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG  
6361 AAATAGACAG ATCGCTGAGA TAGGTGCCTC ACTGATTAAG CATTGGTAAC TGTCAGACCA  
6421 AGTTTACTCA TATATACTTT AGATTGATTT AAAACTTCAT TTTTAATTTA AAAGGATCTA  
6481 GGTGAAGATC CTTTTTGATA ATCTCATGAC CAAAATCCCT TAACGTGAGT TTTCGTTCCA  
6541 CTGAGCGTCA GACCCCGTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG  
6601 CGTAATCTGC TGCTTGCAAA CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTTGCCGGA  
6661 TCAAGAGCTA CCAACTCTTT TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAAA  
6721 TACTGTCCTT CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACCGCC  
6781 TACATACCTC GCTCTGCTAA TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG  
6841 TCTTACCGGG TTGGACTCAA GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC  
6901 GGGGGGTTTCG TGCACACAGC CCAGCTTGA GCGAACGACC TACACCGAAC TGAGATACCT  
6961 ACAGCGTGAG CATTGAGAAA GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC  
7021 GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG  
7081 GTATCTTTAT AGTCCTGTCT GGTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG  
7141 CTCGTCAGGG GGGCGGAGCC TATGGAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT  
7201 GGCCCTTTTGC TGGCCTTTTG CTCACATGTT CTTTCCTGCG TTATCCCTG ATTCTGTGGA  
7261 TAACCGTATT ACCGCCTTTG AGTGAGCTGA TACCGCTCGC CGCAGCCGAA CGACCGAGCG  
7321 CAGCGAGTCA GTGAGCGAGG AAGCGGAAGA GCGCCCAATA CGCAAACCGC CTCTCCCCGC  
7381 GCGTTGGCCG ATTCATTAAT GCAGAGCTTG CAATTCGCGC GTTTTTCAAT ATTATTGAAG  
7441 CATTTATCAG GGTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA  
7501 ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT  
7561 TATTATCATG ACATTAACCT ATAAAAATAG GCGTAGTACG AGGCCCTTTC ACTCATTAGA  
7621 TGCATGTCGT TACATAACTT ACGGTAAATG GCCCGCCTGG CTGACCGCCC AACGACCCCC  
7681 GCCCATTGAC GTCAATAATG ACGTATGTTT CCATAGTAAC GCCAATAGGG ACTTTCCATT  
7741 GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCCACTT GGCAGTACAT CAAGTGTATC  
7801 ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA ATGGCCCGCC TGGCATTATG  
7861 CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG  
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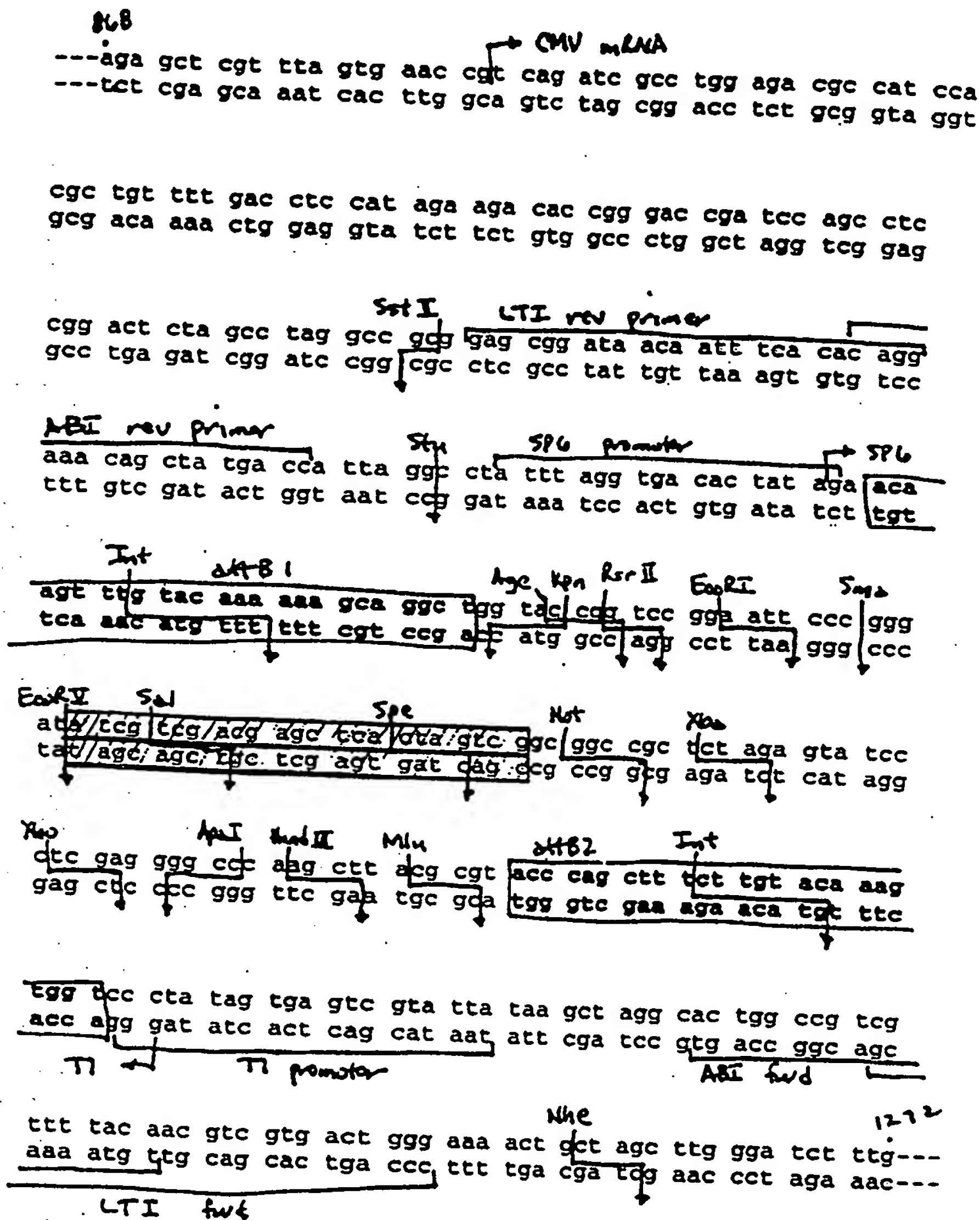


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**Figure 4B A:** pEXP501: pCMV.SPORT 6 host for attB Libraries

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**Figure 4BB:** pEXP501 (cont'd). **Features of the att B cloning vector, pEXP501.** Bases within hatched area are replaced by cDNA in some LTI cDNA libraries.

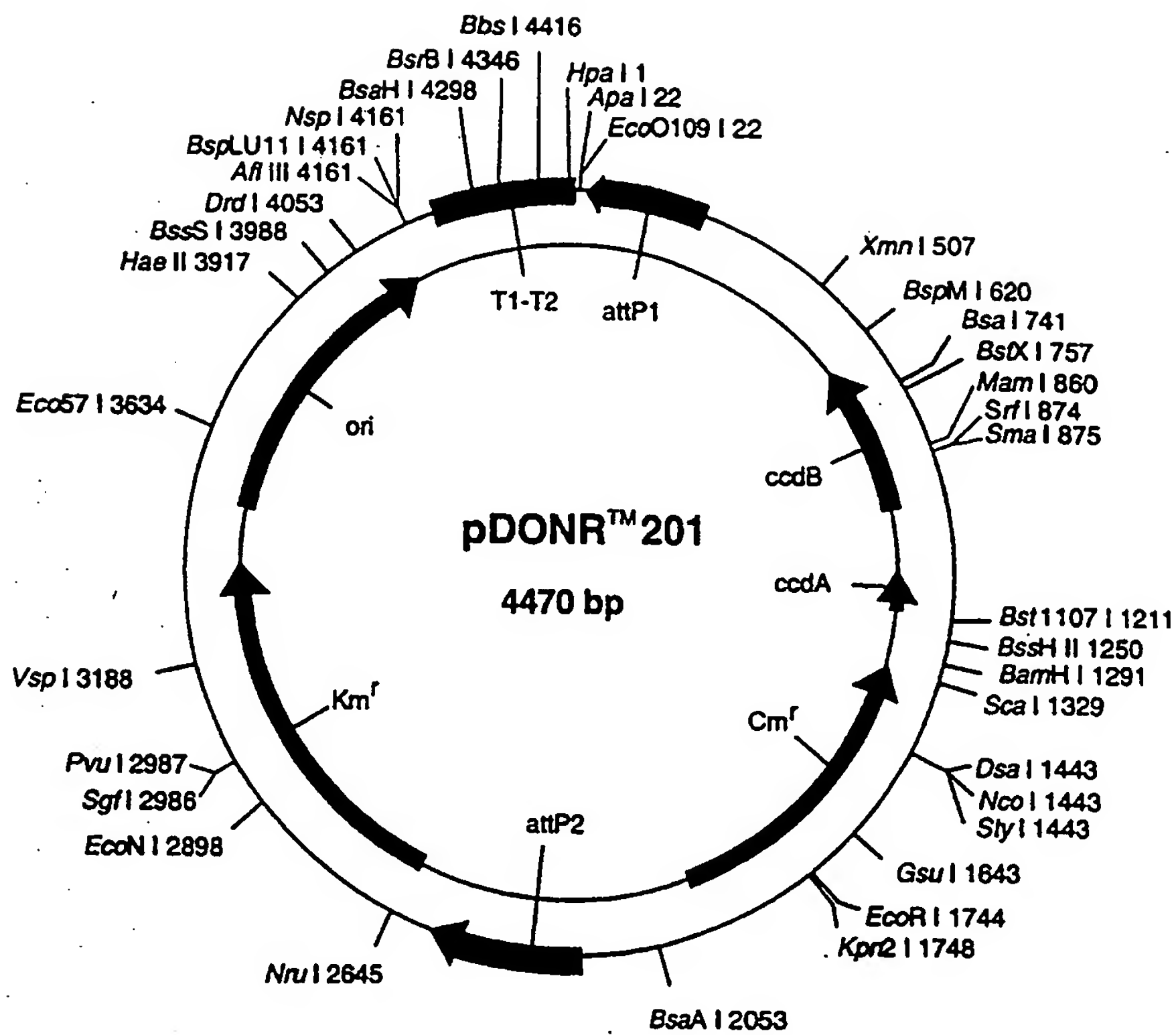


## pEXP501 4396 bp

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61 ATTACGCCAG CCAATACGCA AACCGCCTCT CCCCGCGCGT TGGCCGATTC ATTAATGCAG  
121 GATCGATCCA GACATGATAA GATACATTGA TGAGTTTGGA CAAACCACAA CTAGAATGCA  
181 GTGAAAAAAA TGCTTTATTT GTGAAATTTG TGATGCTATT GCTTTATTTG TAACCATTAT  
241 AAGCTGCAAT AAACAAGTTA ACAACAACAA TTGCATTTCAT TTTATGTTTC AGGTTTCAGGG  
301 GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA AAACCTCTAC AAATGTGGTA TGGCTGATTA  
361 TGATCATGAA CAGACTGTGA GGACTGAGGG GCCTGAAATG AGCCTTGGA CTGTGAATCT  
421 AAAATACACA AACAATTAGA ATCACTAGCT CCTGTGTATA ATATTTTCAT AAATCATACT  
481 CAGTAAGCAA AACTCTCAAG CAGCAAGCAT ATGCAGCTAG TTTAACACAT TATACACTTA  
541 AAAATTTTAT ATTTACCTTA GAGCTTTAAA TCTCTGTAGG TAGTTTGTCC AATTATGTCA  
601 CACCACAGAA GTAAGGTTCC TTCACAAAGA TCCCAAGCTA GCAGTTTTC CAGTCACGAC  
661 GTTGTAAGAC GACGGCCAGT GCCTAGCTTA TAATACGACT CACTATAGGG ACCACTTTGT  
721 ACAAGAAAGC TGGGTACGCG TAAGCTTGGG CCCCTCGAGG GATCCTCTAG AGCGGCCGCC  
781 GACTAGTGAG CTCGTCGACG ATATCCCGGG AATTCGGGAC CGGTACCAGC CTGCTTTTTT  
841 GTACAAACTT GTTCTATAGT GTCACCTAAA TAGGCCTAAT GGTCTAGCT GTTTCCTGTG  
901 TGAAATTGTT ATCCGCTCCG CGGCCTAGGC TAGAGTCCGG AGGCTGGATC GGTCCCGGTG  
961 TCTTCTATGG AGGTCAAAC AGCGTGATG GCGTCTCCAG GCGATCTGAC GGTCACTAA  
1021 ACGAGCTCTG CTTATATAGA CCTCCCACCG TACACGCTA CCGCCCATTT GCGTCAATGG  
1081 GGCGGAGTTG TTACGACATT TTGGAAAGTC CCGTTGATTT TGGTGCCAAA ACAAACTCCC  
1141 ATTGACGTCA ATGGGGTGGA GACTTGAAA TCCCCGTGAG TCAAACCGCT ATCCACGCCC  
1201 ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAATAGCG ATGACTAATA CGTAGATGTA  
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1321 ACCGTCATTG ACGTCAATAG GGGGCGTACT TGGCATATGA TACACTTGAT GTACTGCCAA  
1381 GTGGGCGAGT TACCGTAAAT ACTCCACCCA TTGACGTCAA TGGAAAGTCC CTATTGGCGT  
1441 TACTATGGGA ACATACGTCA TTATTGACGT CAATGGGCGG GGGTCGTTGG GCGGTCAGCC  
1501 AGGCGGGCCA TTTACCGTAA GTTATGTAA GACATGCATC TAATGAGTGA AAGGGCCTCG  
1561 TACTACGCCT ATTTTATAG GTTAATGTCA TGATAATAAT GGTTCCTTAG ACGTCAGGTG  
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1681 ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAACGC  
1741 GCGAATTGCA AGCTCTGCAT TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTCGCGTA  
1801 TTGGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC  
1861 GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG  
1921 CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT  
1981 TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAT CGACGCTCAA  
2041 GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA GGCCTTTCCC CCTGGAAGCT  
2101 CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC  
2161 CTTGCGGAAG CGTGGCGCTT TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG  
2221 TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT  
2281 TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG CCACTGGCAG  
2341 CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG CCGTGCTACA GAGTTCCTGA  
2401 AGTGGTGGCC TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA  
2461 AGCCAGTTAC CTTCCGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG  
2521 GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG  
2581 AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAC TCACGTTAAG  
2641 GGATTTTGGT CATGCCATAA CTTCTGTATG CATACTTAT ACGAAGTTAT GGCATGAGAT  
2701 TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTT AAATCAATCT  
2761 AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA  
2821 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCGT ACTCCCCGTC GTGTAGATAA  
2881 CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC  
2941 GCTCACCAGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA  
3001 GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG  
3061 TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG  
3121 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCAACGA TCAAGGCGAG-

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3961 TGTTAAAATT CGCGTTAAAT TTTTGTTAAA TCAGCTCATT TTTTAACCAA TAGGCCGAAA  
4021 TCGGCAAAAT CCCTTATAAA TCAAAAGAAT AGACCGAGAT AGGGTTGAGT GTTGTTCCAG  
4081 TTTGGAACAA GAGTCCACTA TTAAAGAACG TGGACTCCAA CGTCAAAGGG CGAAAAACCG  
4141 TCTATCAGGG CGATGGCCCA CTACGTGAAC CATCACCTA ATCAAGTTTT TTGGGGTCGA  
4201 GGTGCCGTAA AGCACTAAAT CGGAACCCTA AAGGGAGCCC CCGATTTAGA GCTTGACGGG  
4261 GAAAGCCGGC GAACGTGGCG AGAAAGGAAG GGAAGAAAGC GAAAGGAGCG GCGCTAGGG  
4321 CGCTGGCAAG TGTAGCGGTC ACGCTGCGCG TAACCACCAC ACCCGCCGCG CTTAATGCGC  
4381 CGCTACAGGG CGCGTC

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## pDONR201 4470 bp (rotated to position 3516)

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
260..29	attP1
656..961	ccdB
1099..1184	ccdA
1303..1962	CmR
2210..2442	attP2
2565..3374	Kmr
3495..4134	ori

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1 GTTAACGCTA GCATGGATCT CGGGCCCCAA ATAATGATTT TATTTTGA CT GATAGTGACC
61 TGTTTCGTTGC AACAAATTGA TGAGCAATGC TTTTTTATAA TGCCAACTTT GTACAAAAAA
121 GCTGAACGAG AAACGTAAAA TGATATAAAT ATCAATATAT TAAATTAGAT TTTGCATAAA
181 AAACAGACTA CATAATACTG TAAAACACAA CATATCCAGT CACTATGAAT CAACTACTTA
241 GATGGTATTA GTGACCTGTA GTCGACCGAC AGCCTTCCAA ATGTTCTTCG GGTGATGCTG
301 CCAACTTAGT CGACCGACAG CCTTCCAAAT GTTCTTCTCA AACGGAATCG TCGTATCCAG
361 CCTACTCGCT ATTGTCCTCA ATGCCGTATT AAATCATAAA AAGAAATAAG AAAAAGAGGT
421 GCGAGCCTCT TTTTGTGTG ACAAATAAA AACATCTACC TATTCATATA CGCTAGTGTC
481 ATAGTCCTGA AAATCATCTG CATCAAGAAC AATTTACAAA CTCTTATACT TTTCTCTTAC
541 AAGTCGTTCT GCTTCATCTG GATTTTCAGC CTCTATACTT ACTAAACGTG ATAAAGTTTC
601 TGTAATTTCT ACTGTATCGA CCTGCAGACT GGCTGTGTAT AAGGGAGCCT GACATTTATA
661 TTCCCCAGAA CATCAGGTTA ATGGCGTTTT TGATGTCATT TTCGCGGTGG CTGAGATCAG
721 CCACTTCTTC CCCGATAACG GAGACCGGCA CACTGGCCAT ATCGGTGGTC ATCATGCGCC
781 AGCTTTCATC CCCGATATGC ACCACCGGGT AAAGTTCACG GGAGACTTTA TCTGACAGCA
841 GACGTGCACT GGCCAGGGGG ATCACCATCC GTCGCGCGGG CGTGTCAATA ATATCACTCT
901 GTACATCCAC AAACAGACGA TAACGGCTCT CTCTTTTATA GGTGTAAACC TTAAACTGCA
961 TTTCAACAGT CCCTGTTCTC GTCAGCAAAA GAGCCGTTCA TTTCAATAAA CCGGGCGACC
1021 TCAGCCATCC CTTCCTGATT TTCCGCTTTC CAGCGTTCGG CACGCAGACG ACGGGCTTCA
1081 TTCTGCATGG TTGTGCTTAC CAGACCGGAG ATATTGACAT CATATATGCC TTGAGCAACT
1141 GATAGCTGTC GCTGTCAACT GTCAGTGTA TACGCTGCTT CATAGCACAC CTCTTTTGA
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1261 CGCATACTGT TATCTGGCTT TTAGTAAGCC GGATCCACGC GATTACGCCC CGCCCTGCCA
1321 CTCATCGCAG TACTGTTGTA ATTCAATTAAG CATTCTGCCG ACATGGAAGC CATCACAGAC
1381 GGCATGATGA ACCTGAATCG CCAGCGGCAT CAGCACCTTG TCGCCTTGGC TATAATATTT
1441 GCCCATGGTG AAAACGGGGG CGAAGAAGTT GTCCATATTG GCCACGTTTA AATCAAACT
1501 GGTGAAACTC ACCCAGGGAT TGGCTGAGAC GAAAACATA TTCTCAATAA ACCCTTTAGG
1561 GAAATAGGCC AGGTTTTTAC CGTAACACGC CACATCTTGC GAATATATGT GTAGAACTG
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1681 AACGGTGTA CAAGGGTGAA CACTATCCCA TATCACCAGC TCACCGTCTT TCATTGCCAT
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1801 CTTGTGCTTA TTTTCTTTA CGGTCTTTAA AAAGGCCGTA ATATCCAGCT GAACGGTCTG
1861 GTTATAGGTA CATTGAGCAA CTGACTGAAA TGCCTCAAAA TGTTCTTTAC GATGCCATTG
1921 GGATATATCA ACGGTGGTAT ATCCAGTGAT TTTTCTCTCC ATTTTAGCTT CCTTAGCTCC
1981 TGAAAATCTC GATAACTCAA AAAATACGCC CGGTAGTGAT CTTATTTTCA TATGGTGAAA
2041 GTTGGAACCT CTTACGTGCC GATCAACGTC TCATTTTCGC CAAAAGTTGG CCCAGGGCTT
2101 CCCGGTATCA ACAGGGACAC CAGGATTTAT TTATTCTGCG AAGTGATCTT CCGTCACAGG
2161 TATTTATTCG GCGCAAAGTG CGTCGGGTGA TGCTGCCAAC TTAGTCGACT ACAGGTCACT
2221 AATACCATCT AAGTAGTTGA TTCATAGTGA CTGGATATGT TGTGTTTAC AGTATTATGT
2281 AGTCTGTTTT TTATGCAAAA TCTAATTTAA TATATTGATA TTTATATCAT TTTACGTTTC
2341 TCGTTCAGCT TTCTTGTA CAAGTTGGCAT TATAAGAAAG CATTGCTTAT CAATTTGTTG
2401 CAACGAACAG GTCATATCA GTCAAAATAA AATCATTATT TGCCATCCAG CTGCAGCTCT
2461 GGCCCGTGTC TCAAAATCTC TGATGTTACA TTGCACAAGA TAAAAATATA TCATCATGAA
2521 CAATAAACT GTCTGCTTAC ATAAACAGTA ATACAAGGGG TGTTATGAGC CATATTCAAC
2581 GGGAAACGTC GAGGCCGCGA TTAAATTTCA ACATGGATGC TGATTTATAT GGGTATAAAT
2641 GGGCTCGCGA TAATGTCGGG CAATCAGGTG CGACAATCTA TCGCTGTAT GGGGAGCCCG
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Figure 49B



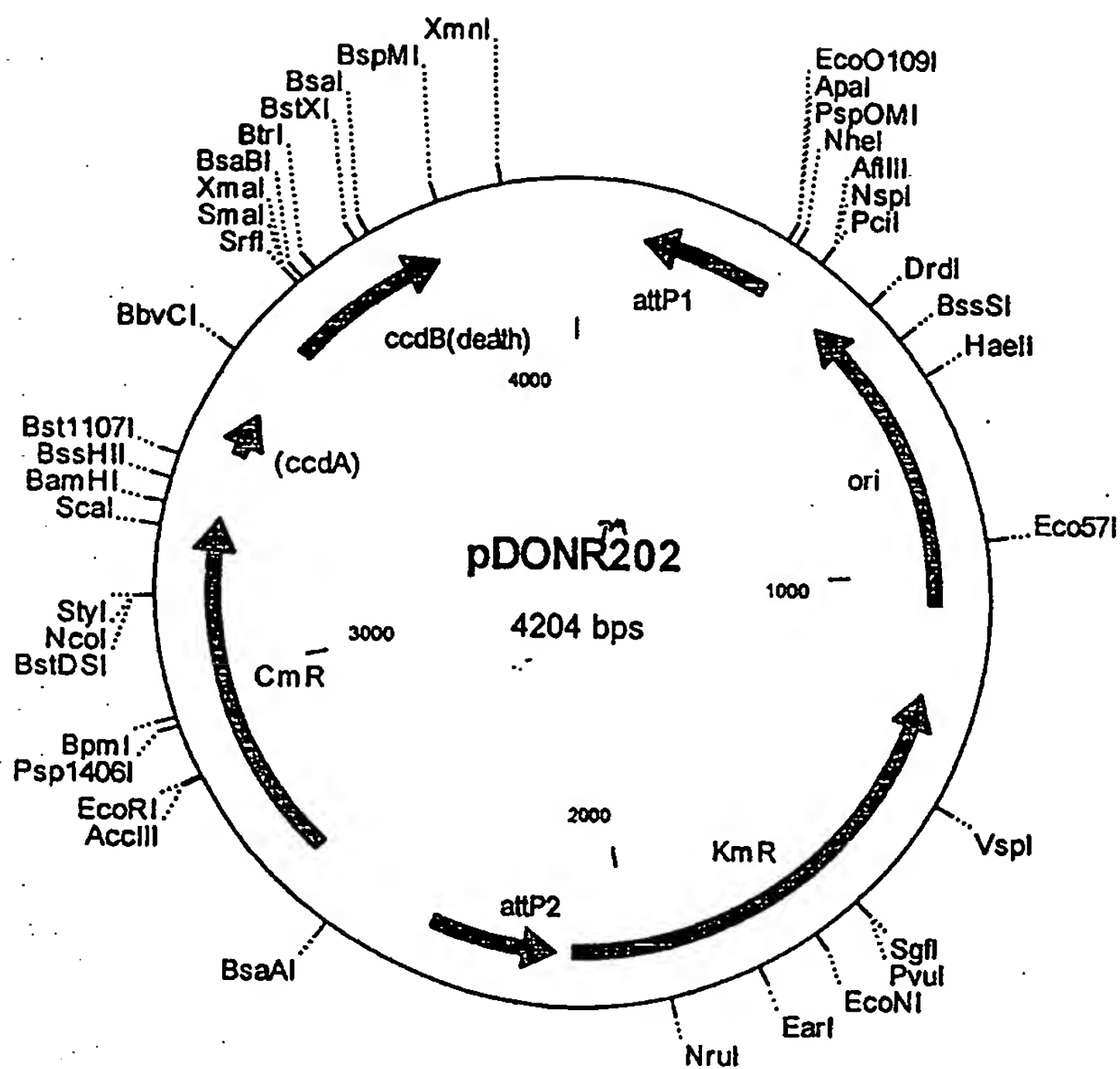
146/240

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3001 GTCTCGCTCA GCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT GATTTTGATG  
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3121 TCTCACC GGA TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTTGTACG  
3181 AGGGGAAATT AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG  
3241 ATCTTGCCAT CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT  
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3361 ATGAGTTTTT CTAATCAGAA TTGGTTAATT GGTGTGAACA CTGGCAGAGC ATTACGCTGA  
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3481 AGCGTCAGAC CCCGTAGAAA AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT  
3541 AATCTGCTGC TTGCAACAA AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA  
3601 AGAGCTACCA ACTCTTTTTC CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC  
3661 TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC  
3721 ATACCTCGCT CTGCTAATCC TGTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT  
3781 TACCGGGTTG GACTCAAGAC GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG  
3841 GGGTTCGTGC ACACAGCCCA GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA  
3901 GCGTGAGCTA TGAGAAAGCG CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT  
3961 AAGCGGCAGG GTCGGAACAG GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA  
4021 TCTTTATAGT CCTGTCGGGT TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC  
4081 GTCAGGGGGG CGGAGCCTAT GGAAAAACGC CAGCAACGCG GCCTTTTTTAC GGTTCCTGGC  
4141 CTTTGTCTGG CCTTTTGCTC ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA  
4201 CCGTATTACC GCTAGCCAGG AAGAGTTTGT AGAAACGCAA AAAGGCCATC CGTCAGGATG  
4261 GCCTTCTGCT TAGTTTGATG CCTGGCAGTT TATGGCGGGC GTCCTGCCCC CCACCCTCCG  
4321 GGCCGTTGCT TCACAACGTT CAAATCCGCT CCCGGCGGAT TTGTCCTACT CAGGAGAGCG  
4381 TTCACCGACA AACACAGAT AAAACGAAAG GCCCAGTCTT CCGACTGAGC CTTTCGTTTT  
4441 ATTTGATGCC TGGCAGTTCC CTACTCTCGC

FIGURE 49C

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FIGURE 50A: pDONR202 (kan<sup>R</sup>)



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## pDONR202 4204 bp

Location (Base Nos.)	Gene Encoded
369..127	attP1
486..1059	ori
1228..2107	KmR
2381..2140	attP2
2629..3288	CmR
3408..3492	inactivated ccdA
3630..3935	ccdB

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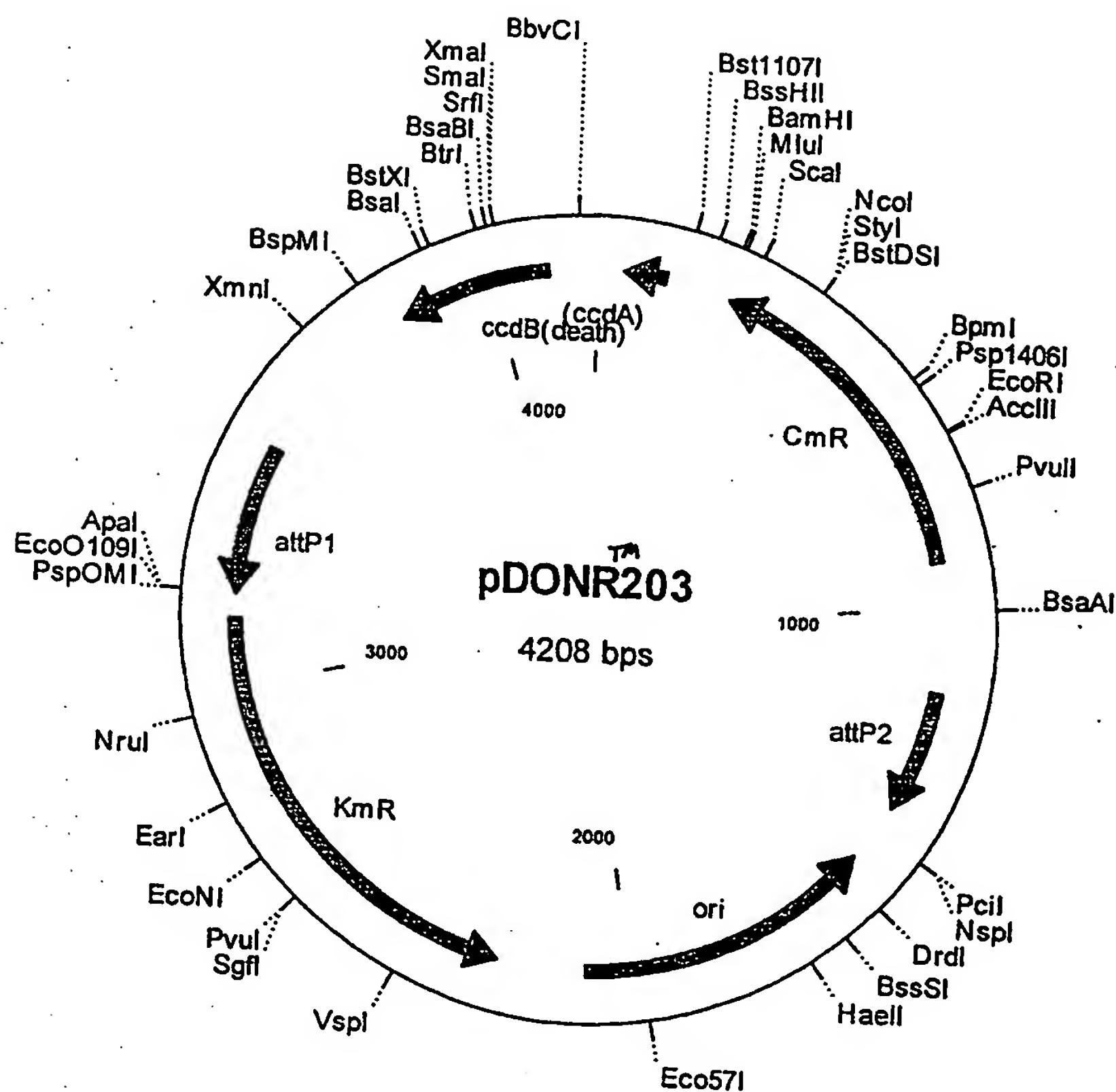
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61 GGAAGGCTGT CGGTCGACTA AGTTGGCAGC ATCACCCGAA GAACATTTGG AAGGCTGTCTG
121 GTCGACTACA GGTCACATAAT ACCATCTAAG TAGTTGATTC ATAGTGACTG GATATGTTGT
181 GTTTTACAGT ATTATGTAGT CTGTTTTTTA TGCAAAATCT AATTTAATAT ATTGATATTT
241 ATATCATTTT ACGTTTCTCG TTCAGCTTTT TTGTACAAAG TTGGCATTAT AAAAAAGCAT
301 TGCTCATCAA TTTGTTGCAA CGAACAGGTC ACTATCAGTC AAAATAAAAT CATTATTTGG
361 GGCCCGAGAT CCATGCTAGC GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA
421 AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG
481 GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG
541 AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG AAGCTCCCTC
601 GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCTT TCTCCCTTCG
661 GGAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT
721 CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTTCAG CCGACCGCTG CGCCTTATCC
781 GGTAACATATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT GGCAGCAGCC
841 ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG
901 TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA
961 GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC
1021 GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT
1081 CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAAAC AAAACTCAGC TTAAGGGATT
1141 TTGGTCATGA GCTTGCGCCG TCCCGTCAAG TCAGCGTAAT GCTCTGCCAG TGTTACAACC
1201 AATTAACCAA TTCTGATTAG AAAAATCAT CGAGCATCAA ATGAAACTGC AATTTATTCA
1261 TATCAGGATT ATCAATACCA TATTTTTGAA AAAGCCGTTT CTGTAATGAA GGAGAAAACT
1321 CACCGAGGCA GTTCCATAGG ATGGCAAGAT CCTGGTATCG GTCTGCGATT CCGACTCGTC
1381 CAACATCAAT ACAACCTATT AATTTCCCCT CGTCAAAAAT AAGGTTATCA AGTGAGAAAT
1441 CACCATGAGT GACGACTGAA TCCGGTGAGA ATGGCAAAAG TTTATGCATT TCTTTCCAGA
1501 CTTGTTCAAC AGGCCAGCCA TTACGCTCGT CATCAAAATC ACTCGCATCA ACCAAACCGT
1561 TATTCATTCTG TGATTGCGCC TGAGCGAGAC GAAATACGCG ATCGCTGTTA AAAGGACAAT
1621 TACAAACAGG AATCGAATGC AACC GGCGCA GGAACACTGC CAGCGCATCA ACAATATTTT
1681 CACCTGAATC AGGATATTCT TCTAATACCT GGAATGCTGT TTTTCCGGGG ATCGCAGTGG
1741 TGAGTAACCA TGCATCATCA GGAGTACGGA TAAATGCTT GATGGTCGGA AGAGGCATAA
1801 ATTCCGTCAG CCAGTTTAGT CTGACCATCT CATCTGTAAC ATCATTGGCA ACGCTACCTT
1861 TGCCATGTTT CAGAAACAAC TCTGGCGCAT CGGGCTTCCC ATACAAGCGA TAGATTGTCTG
1921 CACCTGATTG CCCGACATTA TCGCGAGCCC ATTTATACCC ATATAAATCA GCATCCATGT
1981 TGGAATTTAA TCGCGGCCTC GACGTTTCCC GTTGAATATG GCTCATAACA CCCCTTGAT
2041 TACTGTTTAT GTAAGCAGAC AGTTTATTG TTCATGATGA TATATTTTTA TCTTGTGCAA
2101 TGTAACATCA GAGATTTTGA GACACGGGCC AGAGCTGCAG CTGGATGGCA AATAATGATT
2161 TTATTTTGAC TGATAGTGAC CTGTTGCTTG CAACAAATTG ATAAGCAATG CTTTCTTATA
2221 ATGCCAACTT TGTACAAGAA AGCTGAACGA GAAACGTAAA ATGATATAAA TATCAATATA
2281 TTAAATTAGA TTTTGCATAA AAAACAGACT ACATAATACT GTAAAACACA ACATATCCAG
2341 TCACTATGAA TCAACTACTT AGATGGTATT AGTGACCTGT AGTCGACTAA GTTGGCAGCA
2401 TCACCCGACG CACTTTGCGC CGAATAAATA CCTGTGACGG AAGATCACTT CGCAGAATAA
2461 ATAAATCCTG GTGTCCCTGT TGATACCGGG AAGCCCTGGG CCAACTTTTG GCGAAAATGA
2521 GACGTTGATC GGCACGTAAG AGGTCCAAC TTTCACCATA ATGAAATAAG ATCACTACCG
2581 GGCGTATTTT TTGAGTTATC GAGATTTTCA GGAGCTAAGG AAGCTAAAAT GGAGAAAAAA
2641 ATCACTGGAT ATACCACCGT TGATATATCC CAATGGCATC GTAAAGAACA TTTTGAGGCA
2701 TTTGAGTCAG TTGCTCAATG TACCTATAAC CAGACCGTTC AGCTGGATAT TACGGCCTTT -

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Figure 50B

2761 TTAAAGACCG TAAAGAAAAA TAAGCACAAG TTTTATCCGG CCTTTATTCA CATTCTTGCC  
2821 CGCCTGATGA ATGCTCATCC GGAATTCCGT ATGGCAATGA AAGACGGTGA GCTGGTGATA  
2881 TGGGATAGTG TTCACCCTTG TTACACCGTT TTCCATGAGC AAAC TGAAAC GTTTTCATCG  
2941 CTCTGGAGTG AATACCACGA CGATTTCCGG CAGTTTCTAC ACATATATTC GCAAGATGTG  
3001 GCGTGTTACG GTGAAAACCT GGCCTATTTT CCTAAAGGGT TTATTGAGAA TATGTTTTTC  
3061 GTCTCAGCCA ATCCCTGGGT GAGTTTCACC AGTTTGTGATT TAAACGTGGC CAATATGGAC  
3121 AACTTCTTCG CCCCCGTTTT CACCATGGGC AAATATTATA CGCAAGGCGA CAAGGTGCTG  
3181 ATGCCGCTGG CGATTCAGGT TCATCATGCC GTCTGTGATG GCTTCCATGT CGGCAGAATG  
3241 CTTAATGAAT TACAACAGTA CTGCGATGAG TGGCAGGGCG GGGCGTAATC GCGTGGATCC  
3301 GGCTTACTAA AAGCCAGATA ACAGTATGCG TATTTGCGCG CTGATTTTTG CGGTATAAGA  
3361 ATATATACTG ATATGTATAC CCGAAGTATG TCAAAAAGAG GTGTGCTATG AAGCAGCGTA  
3421 TTACAGTGAC AGTTGACAGC GACAGCTATC AGTTGCTCAA GGCATATATG ATGTCAATAT  
3481 CTCCGGTCTG GTAAGCACAA CCATGCAGAA TGAAGCCCGT CGTCTGCGTG CCGAACGCTG  
3541 GAAAGCGGAA AATCAGGAAG GGATGGCTGA GGTGCCCCGG TTTATTGAAA TGAACGGCTC  
3601 TTTTGCTGAC GAGAACAGGG ACTGGTGAAA TGCAGTTTAA GGTTTACACC TATAAAGAG  
3661 AGAGCCGTTA TCGTCTGTTT GTGGATGTAC AGAGTGATAT TATTGACACG CCCGGGCGAC  
3721 GGATGGTGAT CCCCCTGGCC AGTGCACGTC TGCTGTCAGA TAAAGTCTCC CGTGAACTTT  
3781 ACCCGGTGGT GCATATCGGG GATGAAAGCT GGCGCATGAT GACCACCGAT ATGGCCAGTG  
3841 TGCCGGTCTC CGTTATCGGG GAAGAAGTGG CTGATCTCAG CCACCGCGAA AATGACATCA  
3901 AAAACGCCAT TAACCTGATG TTCTGGGGAA TATAAATGTC AGGCTCCCTT ATACACAGCC  
3961 AGTCTGCAGG TCGATACAGT AGAAATTACA GAAACTTTAT CACGTTTAGT AAGTATAGAG  
4021 GCTGAAAATC CAGATGAAGC CGAACGACTT GTAAGAGAAA AGTATAAGAG TTGTGAAATT  
4081 GTTCTTGATG CAGATGATTT TCAGGACTAT GACACTAGCG TATATGAATA GGTAGATGTT  
4141 TTTATTTTGT CACACAAAAA AGAGGCTCGC ACCTCTTTTT CTTATTTCTT TTTATGATTT  
4201 AATA

FIGURE 51A pDONR203 (kanR)



## pDONR203 4208 bp

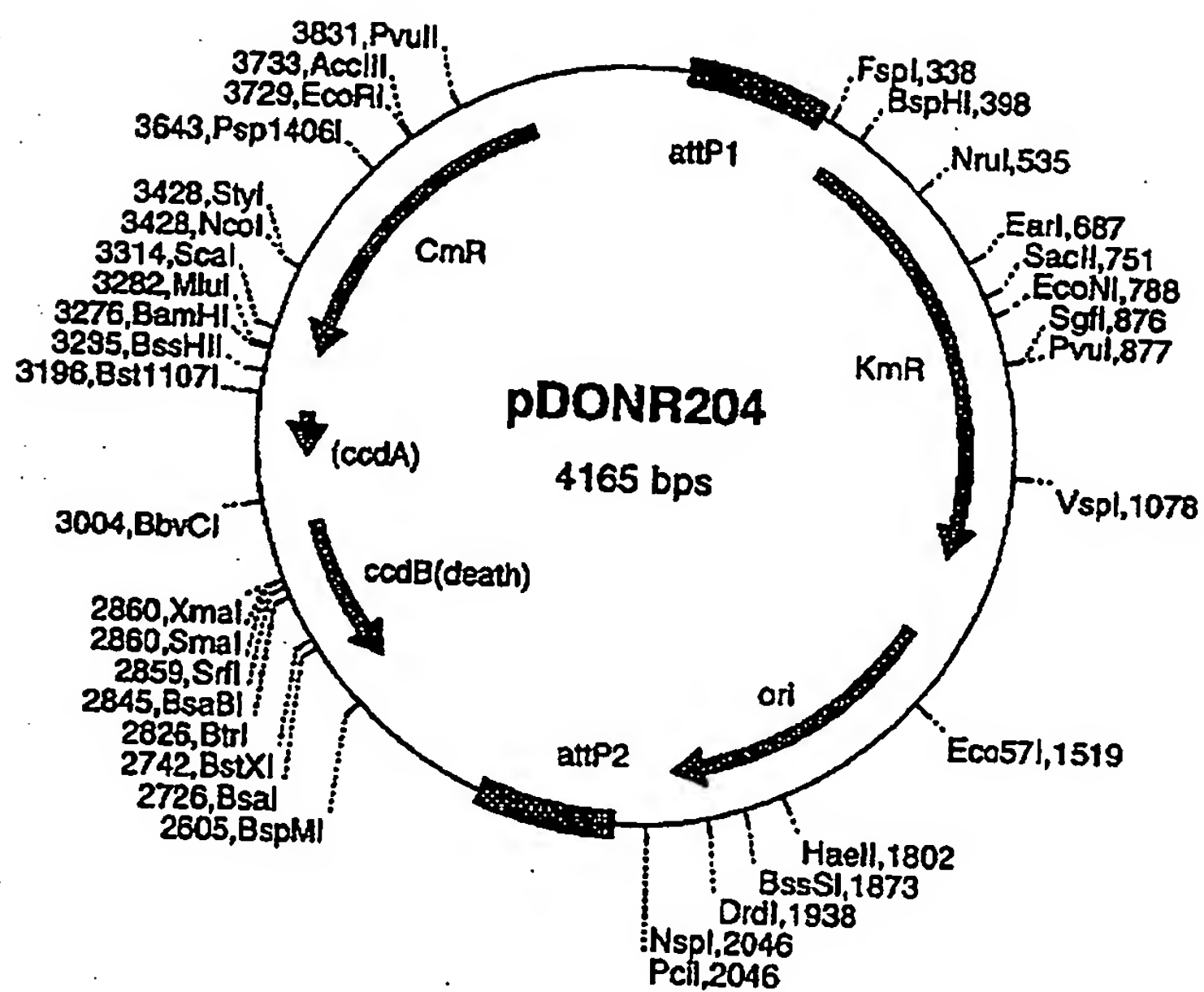
Location (Base Nos.)	Gene Encoded
47..131	inactivated ccdA
251..910	CmR
1158..1398	attP2
1509..2082	ori
2251..3130	KmR
3464..3174	attP1
3812..4117	ccdB

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1 GCGTTCGGCA CGCAGACGAC GGGCTTCATT CTGCATGGTT GTGCTTACCA GACCGGAGAT
61 ATTGACATCA TATATGCCTT GAGCAACTGA TAGCTGTTCG TGTCAACTGT CACTGTAATA
121 CGCTGCTTCA TAGCACACCT CTTTTTGACA TACTTCGGGT ATACATATCA GTATATATTC
181 TTATACCGCA AAAATCAGCG CGCAAATACG CATACTGTTA TCTGGCTTTT AGTAAGCCGG
241 ATCCACGCGT TTACGCCCCG CCCTGCCACT CATCGCAGTA CTGTTGTAAT TCATTAAGCA
301 TTCTGCCGAC ATGGAAGCCA TCACAGACGG CATGATGAAC CTGAATCGCC AGCGGCATCA
361 GCACCTTGTC GCCTTGCGTA TAATATTTGC CCATGGTGAA AACGGGGGCG AAGAAGTTGT
421 CCATATTGGC CACGTTTAAA TCAAACTGG TGAACTCAC CCAGGGATTG GCTGAGACGA
481 AAAACATATT CTCAATAAAC CCTTTAGGGA AATAGGCCAG GTTTTCACCG TAACACGCCA
541 CATCTTGCGA ATATATGTGT AGAACTGCC GGAAATCGTC GTGGTATTCA CTCCAGAGCG
601 ATGAAAACGT TTCAGTTTGC TCATGGAAAA CGGTGTAACA AGGGTGAACA CTATCCCATA
661 TCACCAGCTC ACCGTCTTTC ATTGCCATAC GGAATTCGGG ATGAGCATTG ATCAGGCCGG
721 CAAGAATGTG AATAAAGGCC GGATAAACT TGTGCTTATT TTTCTTTACG GTCCTTAAAA
781 AGGCCGTAAT ATCCAGCTGA ACGGTCTGGT TATAGGTACA TTGAGCAACT GACTGAAATG
841 CCTCAAAATG TTCTTTACGA TGCCATTGGG ATATATCAAC GGTGGTATAT CCAGTGATTT
901 TTTTCTCCAT TTTAGCTTCC TTAGCTCCTG AAAATCTCGA TAACTCAAAA AATACGCCCC
961 GTAGTGATCT TATTTTATTA TGGTGAAAGT TGGAACCTCT TACGTGCCGA TCAACGTCTC
1021 ATTTTCGCCA AAAGTTGGCC CAGGGCTTCC CGGTATCAAC AGGGACACCA GGATTTATTT
1081 ATTCTGCGAA GTGATCTTCC GTCACAGGTA TTTATTGCGC GCAAAGTGCG TCGGGTGATG
1141 CTGCCAACTT AGTCGACTAC AGGTCACTAA TACCATCTAA GTAGTTGATT CATAGTGACT
1201 GGATATGTTG TGTTTTACAG TATTATGTAG TCTGTTTTTT ATGCAAAATC TAATTTAATA
1261 TATTGATATT TATATCATTT TACGTTTCTC GTTCAGCTTT CTTGTACAAA GTTGGCATTG
1321 TAAGAAAGCA TTGCTTATCA ATTTGTTGCA ACGAACAGGT CACTATCAGT CAAAATAAAA
1381 TCATTATTTG CCATCCAGCT AGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA
1441 GGAAAGAACA TGTGAGCAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG
1501 CTGGCGTTTT TCCATAGGCT CCGCCCCCTT GACGAGCATC ACAAATCG ACGCTCAAGT
1561 CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC
1621 CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT
1681 TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC
1741 GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCGTTT AGCCCGACCG CTGCGCCTTA
1801 TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA
1861 GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG
1921 TGGTGGCCTA ACTACGGCTA CACTAGAAGA ACAGTATTTG GTATCTGCGC TCTGCTGAAG
1981 CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT
2041 AGCGGTGGTT TTTTGTGTTG CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA
2101 GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGG ACGAAAACTC ACGTTAAGGG
2161 ATTTTGGTCA TGAGCTTGCG CCGTCCCGTC AAGTCAGCGT AATGCTCTGC CAGTGTTACA
2221 ACCAATTAAC CAATTCTGAT TAGAAAACT CATCGAGCAT CAAATGAAAC TGCAATTTAT
2281 TCATATCAGG ATTATCAATA CCATATTTTT GAAAAAGCCG TTTCTGTAAT GAAGGAGAAA
2341 ACTCACCGAG GCAGTTCCAT AGGATGGCAA GATCCTGGTA TCGGTCTGCG ATTCCGACTC
2401 GTCCAACATC AATACAACCT ATTAATTTCC CCTCGTCAAA AATAAGGTTA TCAAGTGAGA
2461 AATCACCATG AGTGACGACT GAATCCGGTG AGAATGGCAA AAGTTTATGC ATTTCTTTCC
2521 AGACTTGTTT AACAGGCCAG CCATTACGCT CGTCATCAAA ATCACTCGCA TCAACCAAC
2581 CGTTATTTCAT TCGTGATTGC GCCTGAGCGA GACGAAATAC GCGATCGCTG TTAAGAGGAC
2641 AATTACAAAC AGGAATCGAA TGCAACCGGC GCAGGAACAC TGCCAGCGCA TCAACAATAT
2701 TTTCACCTGA ATCAGGATAT TCTTCTAATA CCTGGAATGC TGTTTTTCCG GGGATCGCAG-
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2761 TGGTGAGTAA CCATGCATCA TCAGGAGTAC GGATAAAATG CTTGATGGTC GGAAGAGGCA  
2821 TAAATTCCGT CAGCCAGTTT AGTCTGACCA TCTCATCTGT AACATCATTG GCAACGCTAC  
2881 CTTTGCCATG TTTCAGAAAC AACTCTGGCG CATCGGGCTT CCCATACAAG CGATAGATTG  
2941 TCGCACCTGA TTGCCCCGACA TTATCGCGAG CCCATTTATA CCCATATAAA TCAGCATCCA  
3001 TGTTGGAATT TAATCGCGGC CTCGACGTTT CCCGTTGAAT ATGGCTCATA ACACCCCTTG  
3061 TATTACTGTT TATGTAAGCA GACAGTTTFA TTGTTTATGA TGATATATTT TTATCTTGTTG  
3121 CAATGTAACA TCAGAGATTT TGAGACACGG GCCAGAGCTG CAGCTAGCAT GGATCTCGGG  
3181 CCCCCAATAA TGATTTTATT TTGACTGATA GTGACCTGTT CGTTGCAACA AATTGATGAG  
3241 CAATGCTTTT TTATAATGCC AACTTTGTAC AAAAAAGCTG AACGAGAAAC GTAAAATGAT  
3301 ATAAATATCA ATATATTAAA TTAGATTTTG CATAAAAAAC AGACTACATA ATACTGTAAA  
3361 ACACAACATA TCCAGTCACT ATGAATCAAC TACTTAGATG GTATTAGTGA CCTGTAGTCG  
3421 ACCGACAGCC TTCCAAATGT TCTTCGGGTG ATGCTGCCAA CTTAGTCGAC CGACAGCCTT  
3481 CCAAATGTTC TTCTCAAACG GAATCGTCGT ATCCAGCCTA CTCGCTATTG TCCTCAATGC  
3541 CGTATTAAAT CATAAAAAGA AATAAGAAAA AGAGGTGCGA GCCTCTTTTT TGTGTGACAA  
3601 AATAAAAACA TCTACCTATT CATATACGCT AGTGTCATAG TCCTGAAAAT CATCTGCATC  
3661 AAGAACAATT TCACAACCTCT TATACTTTTC TCTTACAAGT CGTTCGGCTT CATCTGGATT  
3721 TTCAGCCTCT ATACTTACTA AACGTGATAA AGTTTCTGTA ATTTCTACTG TATCGACCTG  
3781 CAGACTGGCT GTGTATAAGG GAGCCTGACA TTTATATTCC CCAGAACATC AGGTTAATGG  
3841 CGTTTTTGAT GTCATTTTCG CGGTGGCTGA GATCAGCCAC TTCTTCCCCG ATAACGGAGA  
3901 CCGGCACACT GGCCATATCG GTGGTCATCA TGCGCCAGCT TTCATCCCCG ATATGCACCA  
3961 CCGGGTAAAG TTCACGGGAG ACTTTATCTG ACAGCAGACG TGCACTGGCC AGGGGGATCA  
4021 CCATCCGTCG CCCGGGCGTG TCAATAATAT CACTCTGTAC ATCCACAAAC AGACGATAAC  
4081 GGCTCTCTCT TTTATAGGTG TAAACCTTAA ACTGCATTTT ACCAGTCCCT GTTCTCGTCA  
4141 GCAAAAGAGC CGTTCATTTT AATAAACCGG GCGACCTCAG CCATCCCTTC CTGATTTTCC  
4201 GCTTTCCA

FIGURE 51C

FIGURE 52A pDONR204 (kan<sup>R</sup>)

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## pDONR204 4165 bp

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1 CGGCATTGAG GACAATAGCG AGTAGGCTGG ATACGACGAT TCCGTTTGAG AAGAACATTT
61 GGAAGGCTGT CGGTCGACTA CAGGTCACCTA ATACCATCTA AGTAGTTGAA TCATAGTGAC
121 TGGATATGTT GTGTTTTTACA GTATTATGTA GTCTGTTTTT TATGCAAAAT CTAATTTAAT
181 ATATTGATAT TTATATCATT TTACGTTTCT CGTTCAGCTT TTTTGTACAA AGTTGGCATT
241 ATAAAAAAGC ATTGCTTATC AATTTGTTGC AACGAACAGG TCACTATCAG TCAAAATAAA
301 ATCATTATTT GGGGCCCCGAG ATCCATGCTA GCTGCAGTGC GCAGGGCCCCG TGTCTCAAAA
361 TCTCTGATGT TACATTGCAC AAGATAAAAA TATATCATCA TGAACAATAA AACTGTCTGC
421 TTACATAAAC AGTAATACAA GGGGTGTTAT GAGCCATATT CAACGGGAAA CGTCTTGCTG
481 GAGGCCGCGA TTAAATTCCA ACATGGATGC TGATTTATAT GGGTATAAAT GGGCTCGCGA
541 TAATGTCGGG CAATCAGGTG CGACAATCTT TCGATTGTAT GGGGAAGCCCC ATGCGCCAGA
601 GTTGTTTCTG AAACATGGCA AAGGTAGCGT TGCCAATGAT GTTACAGATG AGATGGTCAG
661 ACTAAACTGG CTGACGGAAT TTATGCCTCT TCCGACCATC AAGCATTTTA TCCGTACTCC
721 TGATGATGCA TGGTTACTCA CCACTGCGAT CCGCGGGAAA ACAGCATTCG AGGTATTAGA
781 AGAATATCCT GATTCAGGTG AAAATATTGT TGATGCGCTG GCAGTGTTCG TGCGCCGGTT
841 GCATTGATTT CCTGTTTGTA ATTGTCTTTT TAACAGCGAT CGCGTATTTT GTCTCGCTCA
901 GGCGCAATCA CGAATGAATA ACGGTTTGGT TGATGCGAGT GATTTTGATG ACGAGCGTAA
961 TGGCTGGCCT GTTGAACAAG TCTGGAAAGA AATGCATACG CTTTGTCCAT TCTCACCGBA
1021 TTCAGTCGTC ACTCATGGTG ATTTCTCACT TGATAACCTT ATTTTGTACG AGGGGAAATT
1081 AATAGGTTGT ATTGATGTTG GACGAGTCGG AATCGCAGAC CGATACCAGG ATCTTGCCAT
1141 CCTATGGAAC TGCCTCGGTG AGTTTTCTCC TTCATTACAG AAACGGCTTT TTCAAAAATA
1201 TGGTATTGAT AATCCTGATA TGAATAAATT GCAGTTTCAT TTGATGCTCG ATGAGTTTTT
1261 CTAATCAGAA TTGGTTAATT GGTGTGAACA CTGGCAGAGC ATTACGCTGA CTTGACGGGA
1321 CGGCGNCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT
1381 AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTCGTTG CGCGTAATCT GCTGCTTGCA
1441 AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT
1501 TTTTCCGAAG GTAACCTGGT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA
1561 GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACC GCTACATACC TCGCTCTGCT
1621 AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTGGACTC
1681 AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGACACA
1741 GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA
1801 AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG
1861 AACAGGAGAG CGCAGGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT
1921 CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGTA TGCTCGTCAG GGGGGCGGAG
1981 CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT GCTGGCCTTT
2041 TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA TTACCGCTAG
2101 CTGGATCGGC AAATAATGAT TTTATTTTGA CTGATAGTGA CCTGTTCGTT GCAACAAATT
2161 GATAAGCAAT GCTTTTTTAT AATGCCAACT TTGTACAAGA AAGCTGAACG AGAAACGTAA
2221 AATGATATAA ATATCAATAT ATTAAATTAG ATTTTGATAA AAAACAGAC TACATAATAC
2281 TGTAACACAC AACATATCCA GTCACATGTA TTCAACTACT TAGATGGTAT TAGTGACCTG
2341 TAGTCGACTA AGTTGGCAGC ATCACCAGAC GCACTTTGCG CCGAATAAAT ACCTGTGACG
2401 GAAGATCACT TCGCAGAATA AATAAATCCT GGTGTCCCTG TTGATACCGG GAAGCCCTGG
2461 GCCAACTTTT GGCGAAAATG AGACGTTGAT CGGCACATTT CACAACTCTT ATACTTTTCT
2521 CTTACAAGTC GTTCGGCTTC ATCTGGATTT TCAGCCTCTA TACTTACTAA ACGTGATAAA
2581 GTTCTGTGTA TTTCTACTGT ATCGACCTGC AGACTGGCTG TGTATAACGG AGCCTGACAT
2641 TTATATTCCC CAGAACATCA GGTTAATGGC GTTTTGTGAT TCATTTTCGC GGTGGCTGAG
2701 ATCAGCCACT TCTTCCCCGA TAACGGAGAC CGGCACACTG GCCATATCGG TGGTCATCAT
2761 GCGCCAGCTT TCATCCCCGA TATGCACCAC CGGGTAAAGT TCACGGGAGA CTTTATCTGA
2821 CAGCAGACGT GCACTGGCCA GGGGGATCAC CATCCGTCGC CCGGGCGTGT CAATAATATC
2881 ACTCTGTACA TCCACAAACA GACGATAACG GCTCTCTCTT TTATAGGTGT AAACCTTAA
2941 CTGCATTTCA CCAGTCCCTG TTCTCGTCAG CAAAAGAGCC GTTCATTTCA ATAAACCGGG
3001 CGACCTCAGC CATCCCTTCC TGATTTTCCG CTTTCCAGCG TTCGGCACGC AGACGACGGG
3061 CTTCAATCTG CATGGTTGTG CTTACCAGAC CGGAGATATT GACATCATAT ATGCCTTGAG
3121 CAACTGATAG CTGTCGCTGT CAACTGTCAC TGTAATACGC TGCTTCATAG CACACCTCTT
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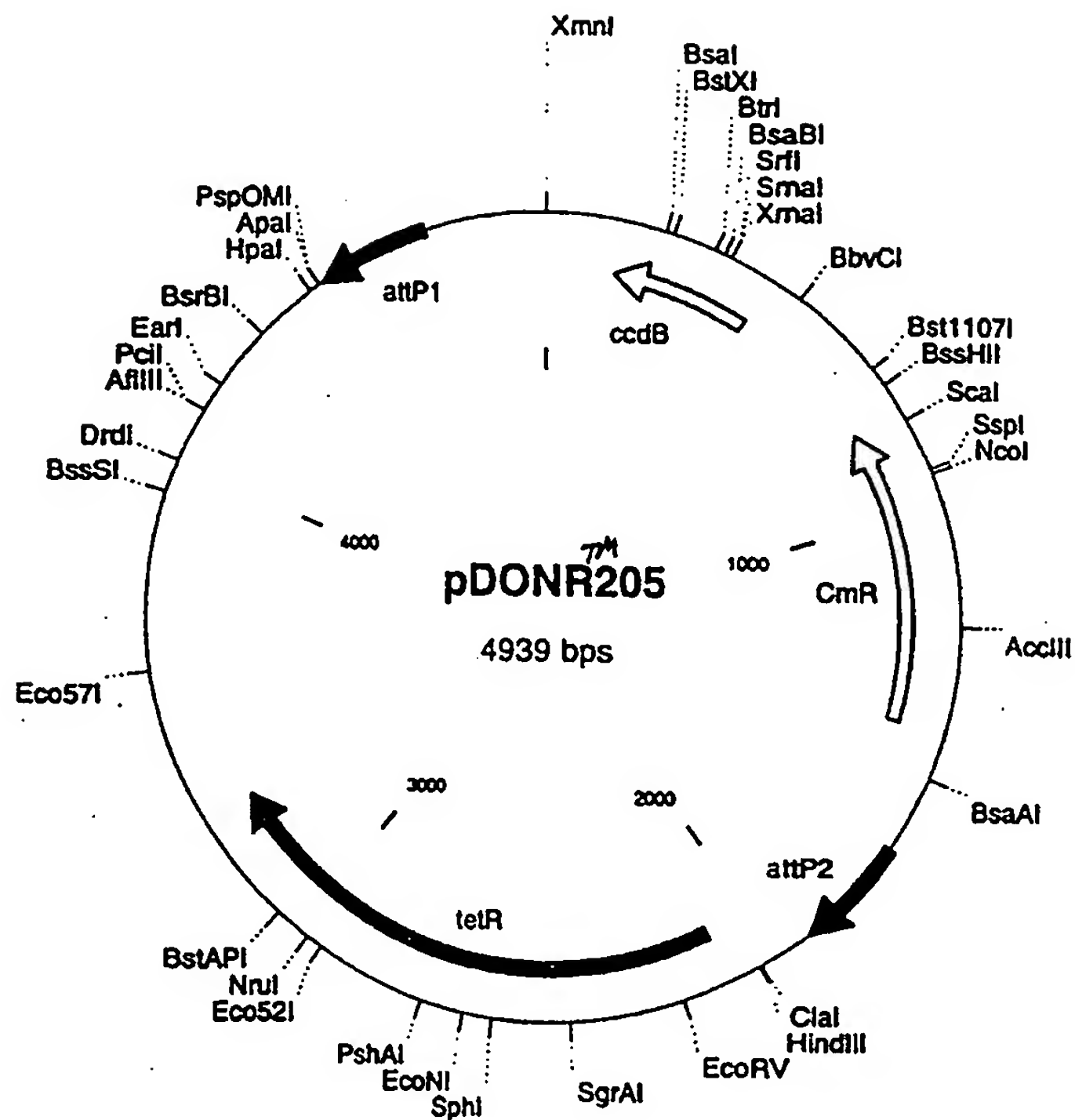
FIGURE 52B

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3241 AAATACGCAT ACTGTTATCT GGCTTTTAGT AAGCCGGATC CACGCGTTTA CGCCCCGCCC  
3301 TGCCACTCAT CGCAGTACTG TTGTAATTCA TTAAGCATTG TGCCGACATG GAAGCCATCA  
3361 CAGACGGCAT GATGAACCTG AATCGCCAGC GGCATCAGCA CTTGTGCGCC TTGCGTATAA  
3421 TATTTGCCCA TGGTGAAAAC GGGGGCGAAG AAGTTGTCCA TATTGGCCAC GTTTAAATCA  
3481 AACTGGTGGA AACTCACCCA GGGATTGGCT GAGACGAAAA ACATATTCTC AATAAACCTT  
3541 TTAGGGAAAT AGGCCAGGTT TTCACCGTAA CACGCCACAT CTTGCGAATA TATGTGTAGA  
3601 AACTGCCGGA AATCGTCGTG GTATTCACTC CAGAGCGATG AAAACGTTTC AGTTTGCTCA  
3661 TGGAAAACGG TGTAACAAGG GTGAACACTA TCCCATATCA CCAGCTCACC GTCTTTCATT  
3721 GCCATACGGA ATTCCGGATG AGCATTTCATC AGGCGGGCAA GAATGTGAAT AAAGGCCGGA  
3781 TAAAACCTGT GCTTATTTTT CTTTACGGTC TTTAAAAGG CCGTAATATC CAGCTGAACG  
3841 GTCTGGTTAT AGGTACATTG AGCAACTGAC TGAAATGCCT CAAAATGTTC TTTACGATGC  
3901 CATTGGGATA TATCAACGGT GGTATATCCA GTGATTTTTT TCTCCATTTT AGCTTCCTTA  
3961 GCTCCTGAAA ATCTCGATAA CTCAAAAAT ACGCCCGGTA GTGATCTTAT TTCATTATGG  
4021 TGAAAGTTGG AACCTCTTAC TGTTCCTTGAT GCAGATGATT TTCAGGACTA TGACACTAGC  
4081 ATATATGAAT AGGTAGATGT TTTTATTTTG TCACACAAAA AAGAGGCTCG CACCTCTTTT  
4141 TCTTATTTCT TTTTATGATT TAATA

FIGURE 52C

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Figure 53A: pDONR205 (tetR)



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pDONR205 4939 bp

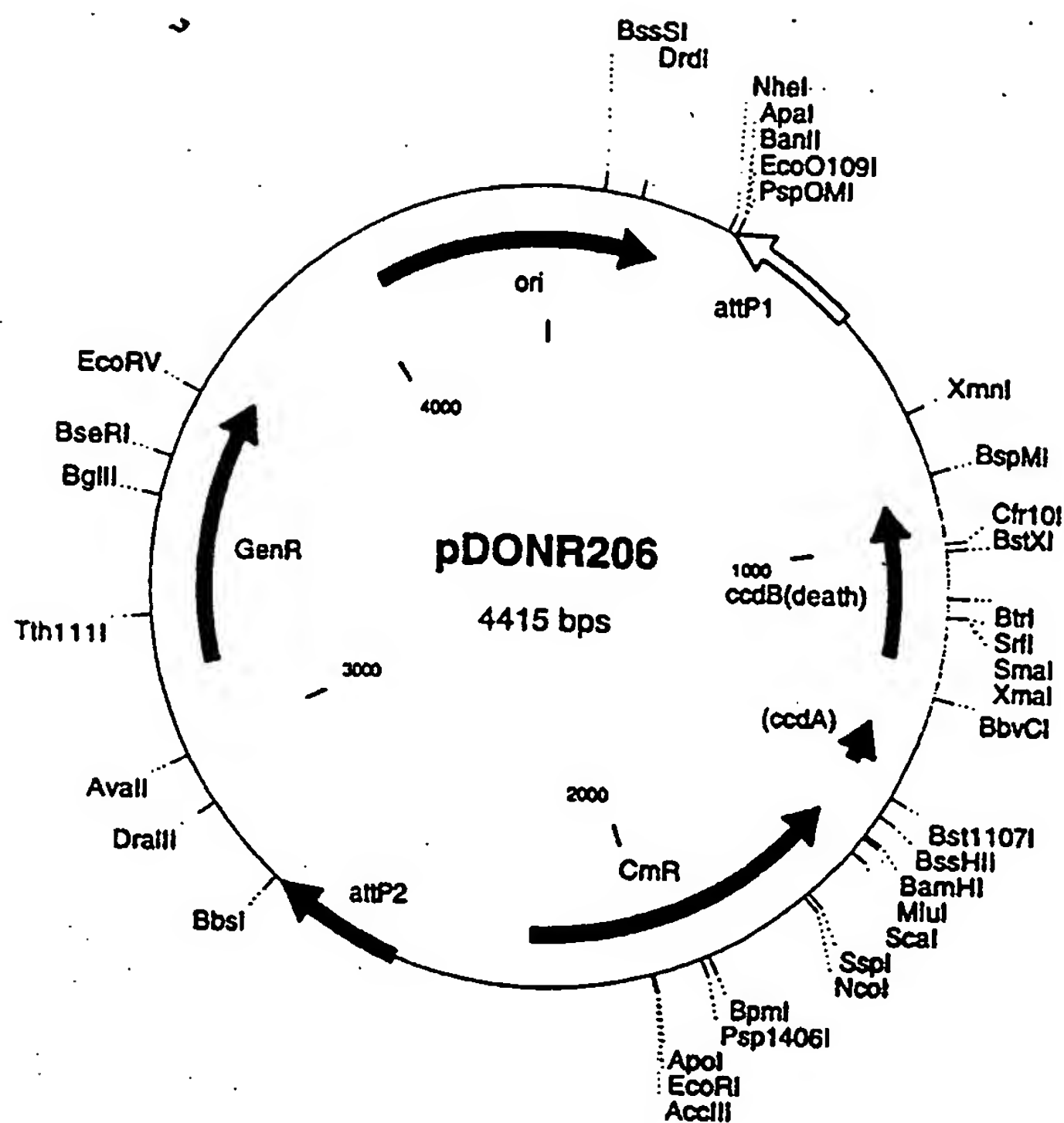
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GGCATTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAA  
AATAAAATCATTATTTGCCATCCAGCTGCAGCTCTGGCCCGTGTCTCAAAATCTCTGATG  
TTACATTGCACAAGATAAAAAATATATCATCATGAATTTCTCATGTTTGACAGCTTATCATC  
GATAAGCTTTAATGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGGCACCGTGT  
ATGAAATCTAACAATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTGTAGGC  
ATAGGCTTGTTATGCGGTAAGTGCAGGCTCTGCGGGGCTCTTGGCGGATATCGTCCATTCCGACAGC  
ATCGCCAGTCACTATGGCGTGTGCTAGCGCTATATGCGTTGATGCAATTTCTATGCGCA  
CCCGTTCTCGGAGCACTGTCCGACCGCTTGGCCGCGCCAGTCTGCTCGCTTCGCTA  
CTTGAGCCACTATCGACTACGCGATCATGGCGACCACACCCGTCCTGTGGATCCTCTAC  
GCCGGACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATC  
GCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTC  
GGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTGTTGGGCGCCATCTCCTTGCA  
GCACCATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTA  
ATGCAGGAGTGCATAAGGGAGAGCGTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTC  
AGCTCCTTCCGGTGGGCGCGGGCATGACTATCGTCGCCGCACTTATGACTGTCTTCTTT  
ATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTTTCGGCGAGGACCGC  
TTTCGCTGGAGCGCGACGATGATCGGCCTGTCGCTTGCGGTATTCGGAATCTTGACGCGC  
CTCGCTCAAGCCTTCGTCACTGGTCCCGCCACCAAACGTTTCGGCGAGAAGCAGGCCATT  
ATCGCCGGCATGGCGGCCGACGCGCTGGGCTACGTCTTGCTGGCGTTTCGCGACGCGAGGC  
TGGATGGCCTTCCCATTATGATTCTTCTCGCTTCCGGCGGCATCGGGATGCCCGCGTTG  
CAGGCCATGCTGTCCAGGCAGGTAGATGACGACCATCAGGGACAGCTTCAAGGATCGCTC  
GCGGCTCTTACCAGCCTAACTTCGATCATTGGACCGCTGATCGTCACGGCGATTTATGCC  
GCCTCGGCGAGCACATGGAACGGGTTGGCATGGATTGTAGGCGCCGCCCTATACCTTGTC  
TGCTTCCCCGCGTTGCGTCCGGTGATGGAGCCGGGCCACCTCGACCTGAATGGAAGCC  
GGCGGCACCTCGCTAACGGATTCAACACTCCAAGAATTGGAGCCAATCAATTCTTGCGGA  
GAACTGTGAATGCGCAAACCAACCCTTGGCAGAACATATCCATCGCATGACCAAAATCCC  
TTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTC  
TTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAACACCGCTACC  
AGCGGTGGTTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTT  
CAGCAGAGCGCAGATACCAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTT  
CAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGC  
TGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAA  
GGCGCAGCGGTGCGGCTGAACGGGGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGAC  
CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGG  
GAGAAAGGCGGACAGGTATCCGTAAGCGGCAGGTCGGAACAGGAGAGCGCACGAGGGA  
GCTTCCAGGGGGAACGCTGATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACT  
TGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAACGCGCAGCAA-

FIGURE 53B



CGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGC  
GTTATCCCCTGATTCTGTGGATAACCGTATTACCGCTAGCCAGGAAGAGTTTGTAGAAAC  
GCAAAAAGGCCATCCGTCAGGATGGCCTTCTGCTTAGTTTGATGCCTGGCAGTTTATGGC  
GGGCGTCTGCCCCGCCACCCTCCGGGCCGTTGCTTCAACAGTTCAAATCCGCTCCCGGC  
GGATTGTCTACTCAGGAGAGCGTTCACCGACAAACAACAGATAAAACGAAAGGCCAG  
TCTTCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCCCTACTCTCGCGTTAAC  
GCTAGCATGGATCTCGGGCCCCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTTCG  
TTGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAACTTTGTACAAAAAGCTGAA  
CGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAACAG  
ACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATGGT  
ATTAGTGACCTGTAGTCGACCGACAGCCTTCCAAATGTTCTTCGGGTGATGCTGCCAACT  
TAGTCGACCGACAGCCTTCCAAATGTTCTTCTCAAACGGAATCGTCGTATCCAGCCTACT  
CGCTATTGTCTCAATGCCGTATTAAATCATAAAAAGAAATAAGAAAAAGAGGTGCGAGC  
CTCTTTTTTGTGTGACAAAATAAAAAACATCTACCTATTATATACGCTAGTGTCATAGTC  
CTGAAAATCATCTGCATCAAGAACAATTTCACAACTCTTATACTTTTCTTTACAAGTCG  
TTCGGCTTCATCTGGATTTTCAGCCTCTATACTTACTAAACGTGATAAAGTTTCTGTAAT  
TTCTACTGTATCGACCTGCAGACTGGCTGTGTATAAGGGAGCCTGACATTTATATTCCCC  
AGAACATCAGGTTAATGGCGTTTTTGTATGTCATTTTCGCGGTGGCTGAGATCAGCCACTT  
CTTCCCCGATAACGGAGACCGGCACACTGGCCATATCGGTGGTCATCATGCGCCAGCTTT  
CATCCCCGATATGCACCACCGGGTAAAGTTCACGGGAGACTTATCTGACAGCAGACGTG  
CACTGGCCAGGGGGATCACCATCCGTCGCCCCGGCGTGTCAATAATATCACTCTGTACAT  
CCACAAACAGACGATAACGGCTCTCTCTTTTATAGGTGTAAACCTTAAACTGCATTTTAC  
CAGTCCCTGTTCTCGTCAGCAAAAGAGCCGTTCAATTTCAATAAACGGGCGACCTCAGCC  
ATCCCTTCTGATTTTCCGCTTTCAGCGTTCGGCACGCAGACGACGGGCTTCATTCTGC  
ATGGTTGTGCTTACCAGACCGGAGATATTGACATCATATATGCCTTGAGCAACTGATAGC  
TGTCGCTGTCAACTGTCACTGTAATACGCTGCTTCATAGCACACCTCTTTTGTACATACT  
TCGGGTATACATATCAGTATATATTCTTATACCGCAAAAATCAGCGCGCAAAATACGCATA  
CTGTTATCTGGCTTTTAGTAAGCCGGATCCACGCGATTACGCCCCGCCCTGCCACTCATC  
GCAGTACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAGACGGCATG  
ATGAACCTGAATCGCCAGC

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## pDONR206 4415 bp

CGGCATTGAGGACAATAGCGAGTAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTT  
GGAAGGCTGTCGGTCTGACTACAGGTCACTAATACCATCTAAGTAGTTGAATCATAGTGAC  
TGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAAT  
ATATTGATATTTATATCATTTTACGTTTCTCGTTTCAGCTTTTTTTGTACAAAGTTGGCATT  
ATAAAAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCACTATCAGTCAAAATAAA  
ATCATTATTTGGGGCCCGAGATCCATGCTAGCGGTAATACGGTTATCCACAGAATCAGGG  
GATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAG  
GCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGA  
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT  
GGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCC  
TTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCTG  
GTGTAGGTCTGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCGACCGC  
TGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA  
CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAG  
TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT  
CTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACC  
ACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGA  
TCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAACTCA  
CGTTAAGGGATTTTGGTTCATGNCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGT  
TACAACCAATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAAACTGCAAT  
TTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGA  
GAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCG  
ACTCGTCCAACATCAATACAACCTATTAGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGC  
AGATCCGTGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCCAATGCCTGACGATGC  
GTGGAGACCGAAACCTTGCGCTCGTTCCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTG  
CTGCCCAAGGTTGCCGGGTGACGCACACCGTGGAACGGATGAAGGCACGAACCCAGTTG  
ACATAAGCCTGTTCCGTTTCGTAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGG  
TCCAGAACCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGT  
TATGACTGTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCC  
GTGGGTGATGTTTGTATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTAC  
GCAGCAGGGCAGTCGCCCTAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTGCGAC  
ATGTAGGCTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCTG  
TGAGTTCGGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAA  
CTTGCTCCGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGG  
CGCTCTCGCGGCTTACGTTCTGCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTA  
TGATCTCGCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCT  
CCTCAAGCATGAGGCCAACGCGCTTGGTGCTTATGTGATCTACGTGCAAGCAGATTACGG  
TGACGATCCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTT  
TGATATCGACCCAAGTACCGCCACCTAACAATTCGTTCAAGCCGAGATCGGCTTCCCGGC  
CTAATTTCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTG  
AATCCGGTGAGAATGGCAAAAGCGTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGC  
CATTACGCTCGTCATCAAAATCACTCGCATCAACCAACCGTTATTTCATTCTGTGATTGCG  
CCTGAGCGAGACGAAATACGCGATCGCTGTTAAAGGACAATTACAAACAGGAATCGAAT  
GCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATT  
CTTCTAATACCTGGAATGCTGTTTTCCCGCGGATCGCAGTGGTGAGTAACCATGCATCAT  
CAGGAGTACGGATAAAATGCTTGATGGTCCGAAGAGGCATAAATCCGTCAGCCAGTTTA  
GTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACA  
ACTCTGGCGCATCGGGCTTCCCATACAATCGAAAGATTGTCGCACCTGATTGCCCGACAT  
TATCGCGAGCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCC  
TCCAGCAAGACGTTTTCCCGTTGAATATGGCTCATAACACCCCTTGATTACTGTTTATGT  
AAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTCATGTAACATCAGA  
GATTTTGAGACACGGGCCCNGCGCACTGCAGCTGGATCGGCAATAATGATTTTATTTTG  
ACTGATAGTGACCTGTTTCGTTGCAACAAATTGATAAGCAATGCTTTTTTATAATGCCAAC -

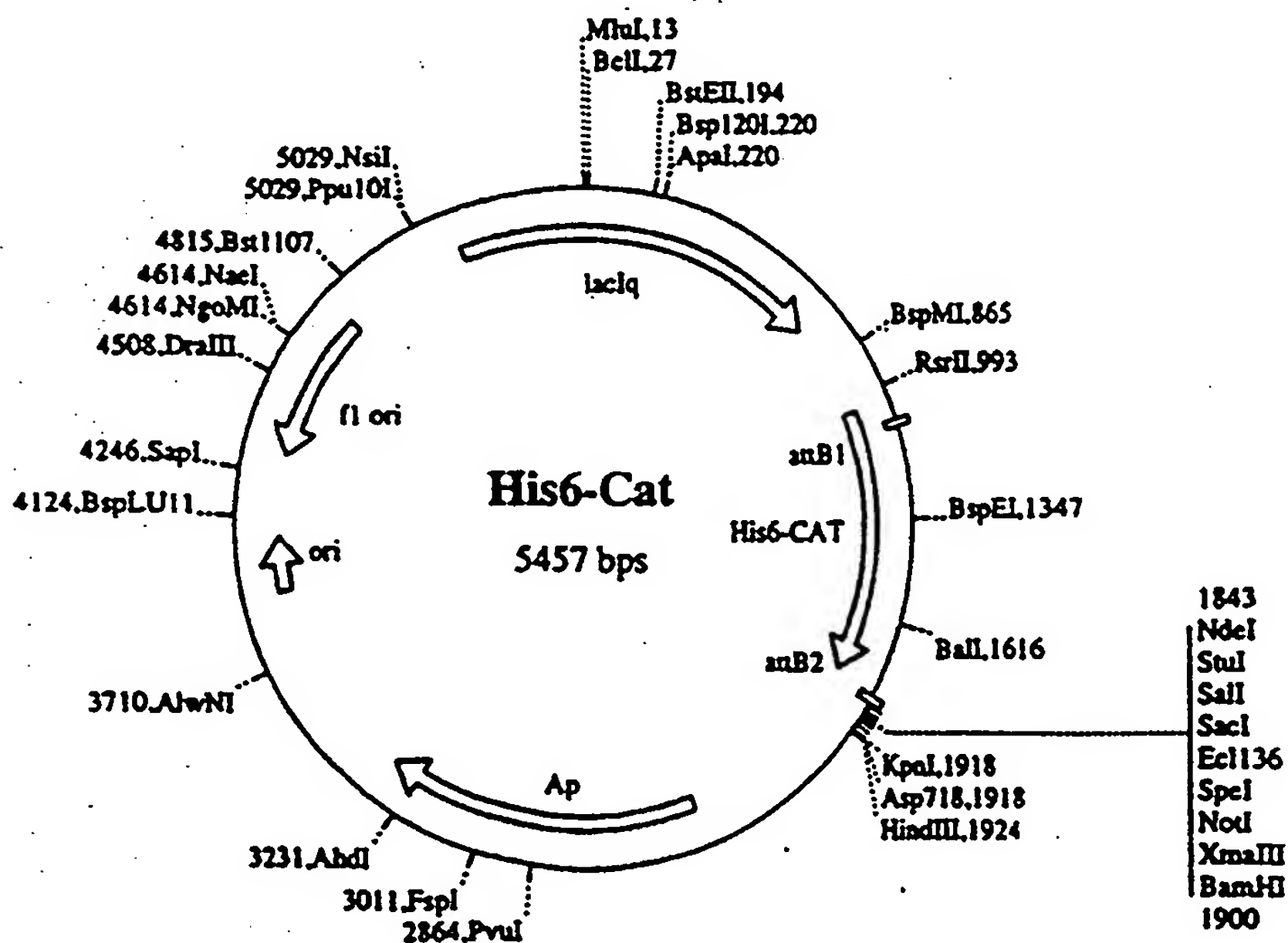
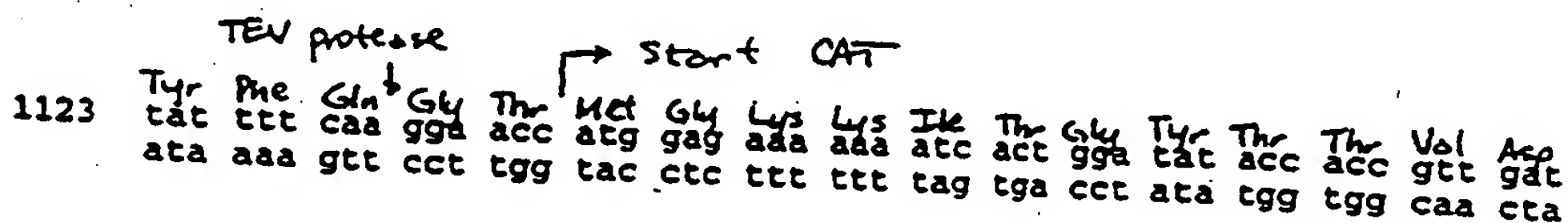
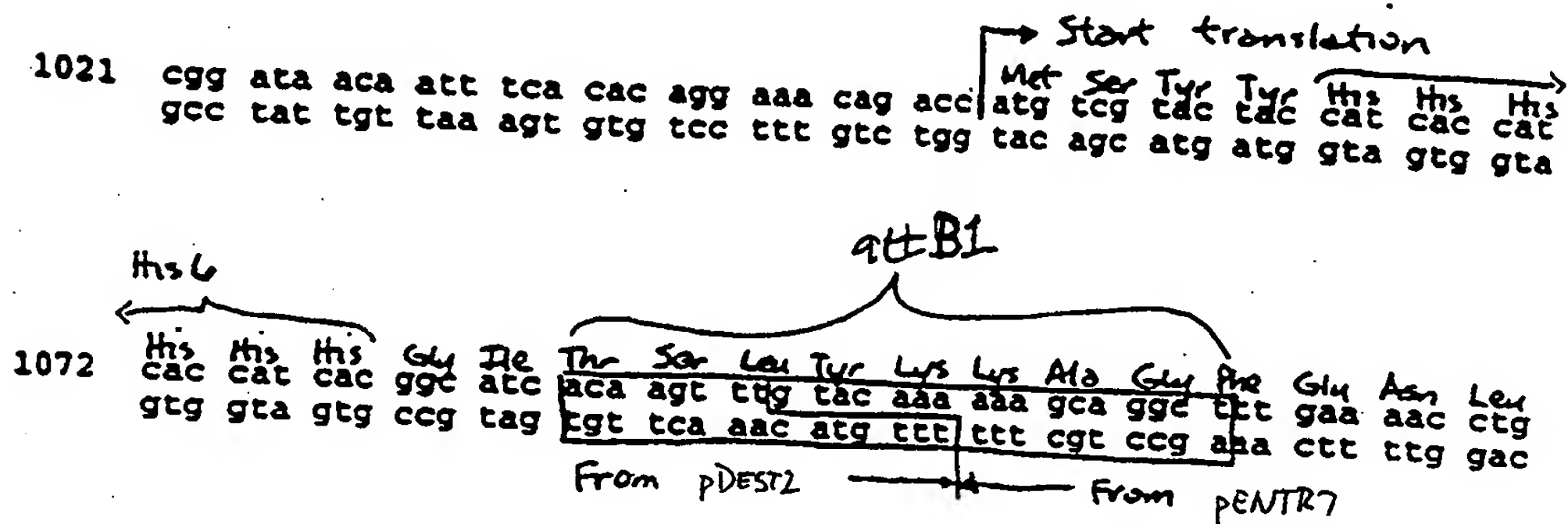
FIGURE 54B

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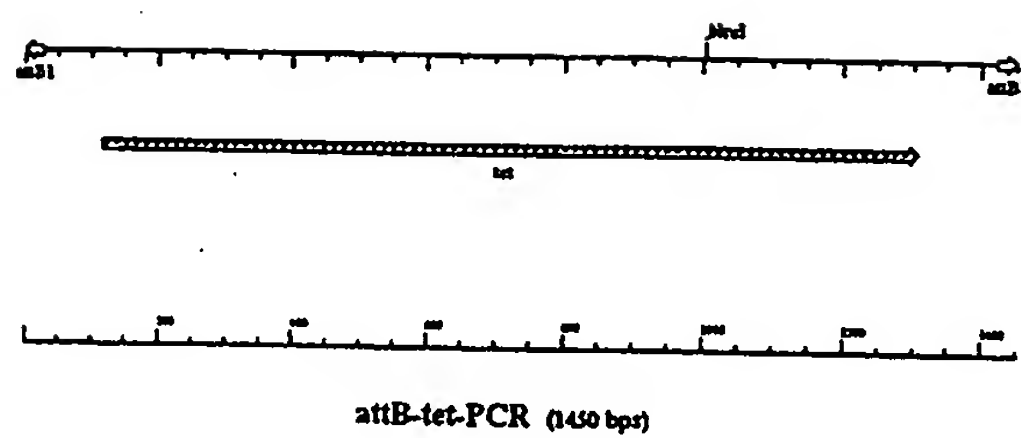
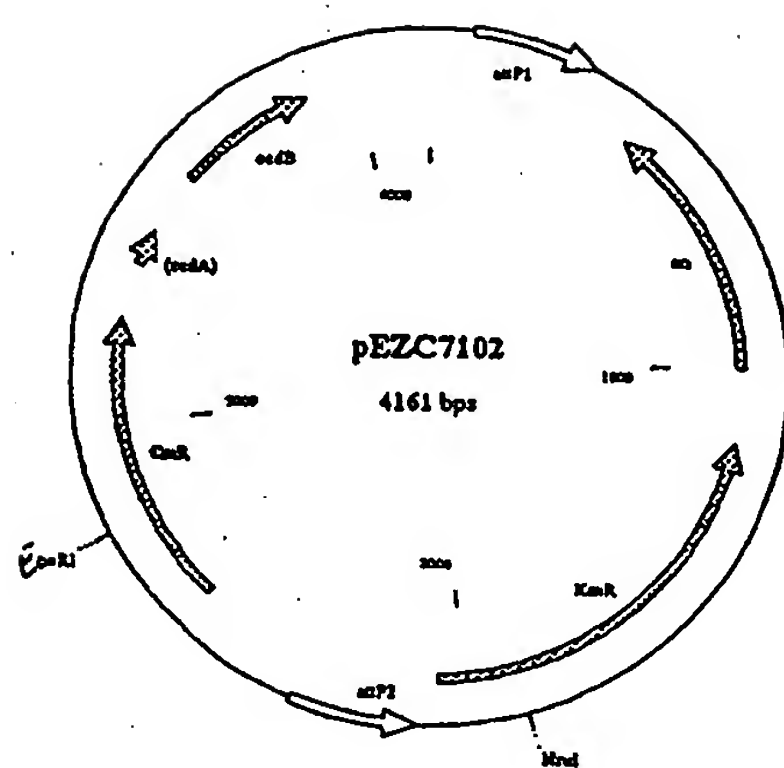
TTTGTACAAGAAAGCTGAACGAGAAACGTAAATGATATAAATATCAATATATTAAATTA  
GATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACATATCCAGTCACTATG  
ATTCAACTACTTAGATGGTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGA  
CGCACTTTGCGCCGAATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCC  
TGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGA  
TCGGCACGTAAGAGGTTCCAACCTTCACCATAATGAAATAAGATCACTACCGGGCGTATT  
TTTTGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAATGGAGAAAAAATCACTGG  
ATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTC  
AGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTAAAGAC  
CGTAAAGAAAAATAAGCACAGTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGAT  
GAATGCTCATCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAG  
TGTTCAACCCTTGTTACACCGTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAG  
TGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTA  
CGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGC  
CAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTT  
CGCCCCCGTTTTTACCATGGGCAAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCT  
GGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGA  
ATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACCGCTGGATCCGGCTTACT  
AAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATAC  
TGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTG  
ACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTC  
TGGTAAGCACAAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGG  
AAAATCAGGAAGGGATGGCTGAGGTGCGCCGGTTTTATTGAAATGAACGGCTCTTTTGCTG  
ACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGGTTTACACCTATAAAAGAGAGAGCCGT  
TATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTG  
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GTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTC  
TCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCC  
ATTAACCTGATGTTCTGGGGAATATAAATGTGAGGCTCCGTTATACACAGCCAGTCTGCA  
GGTCGATACAGTAGAAATTACAGAACTTTATCACGTTTAGTAAGTATAGAGGCTGAAAA  
TCCAGATGAAGCCGAACGACTTGTAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGA  
TGCAGATGATTTTCAGGACTATGACACTAGCATATATGAATAGGTAGATGTTTTTATTTT  
GTCACACAAAAAGAGGCTCGCACCTCTTTTCTTATTTCTTTTATGATTTAATA

FIGURE 54C

Figure 55 An Entry (pENTR7) Clone of CAT Subcloned into pDEST2



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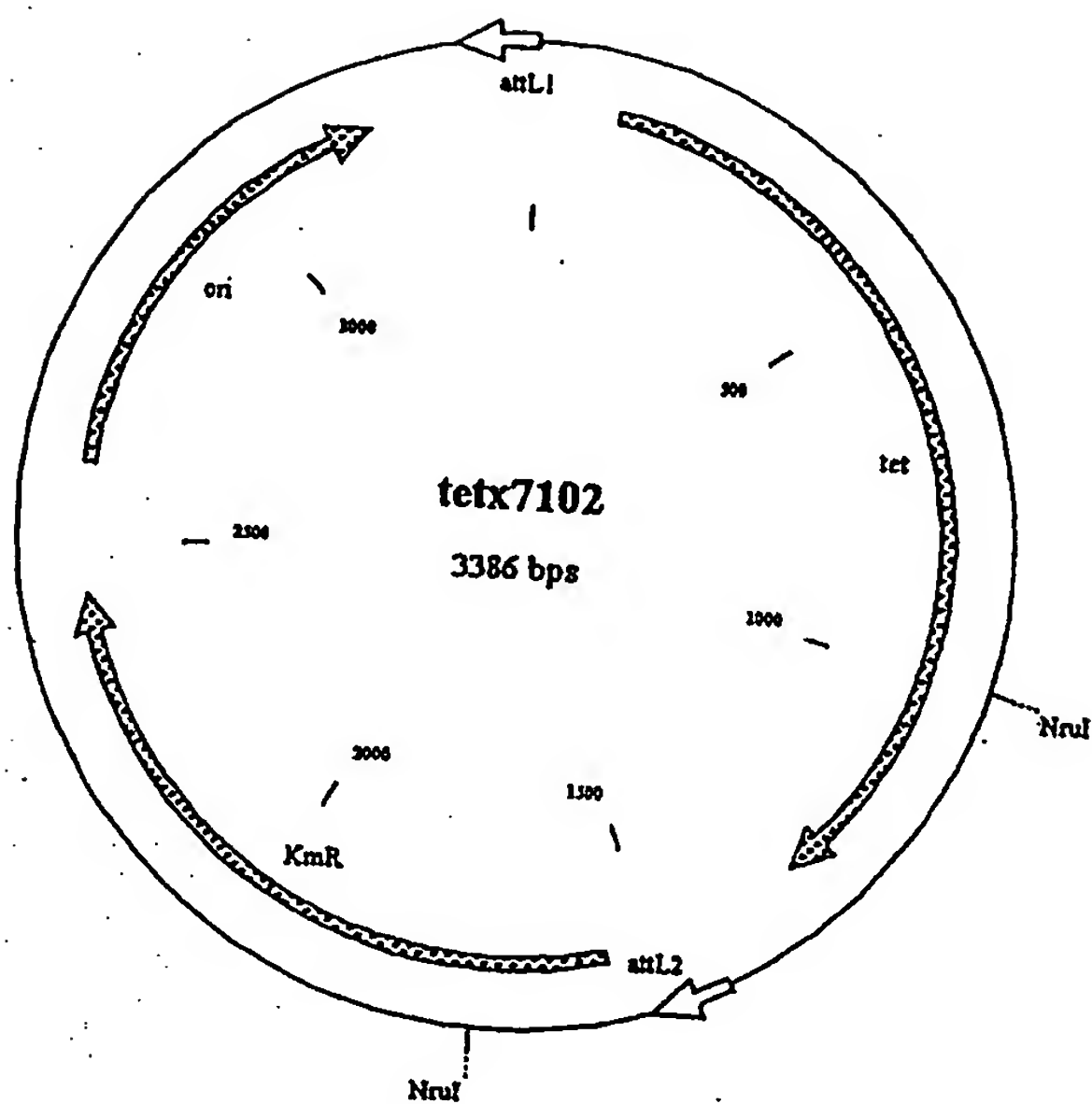


FIGURE 57

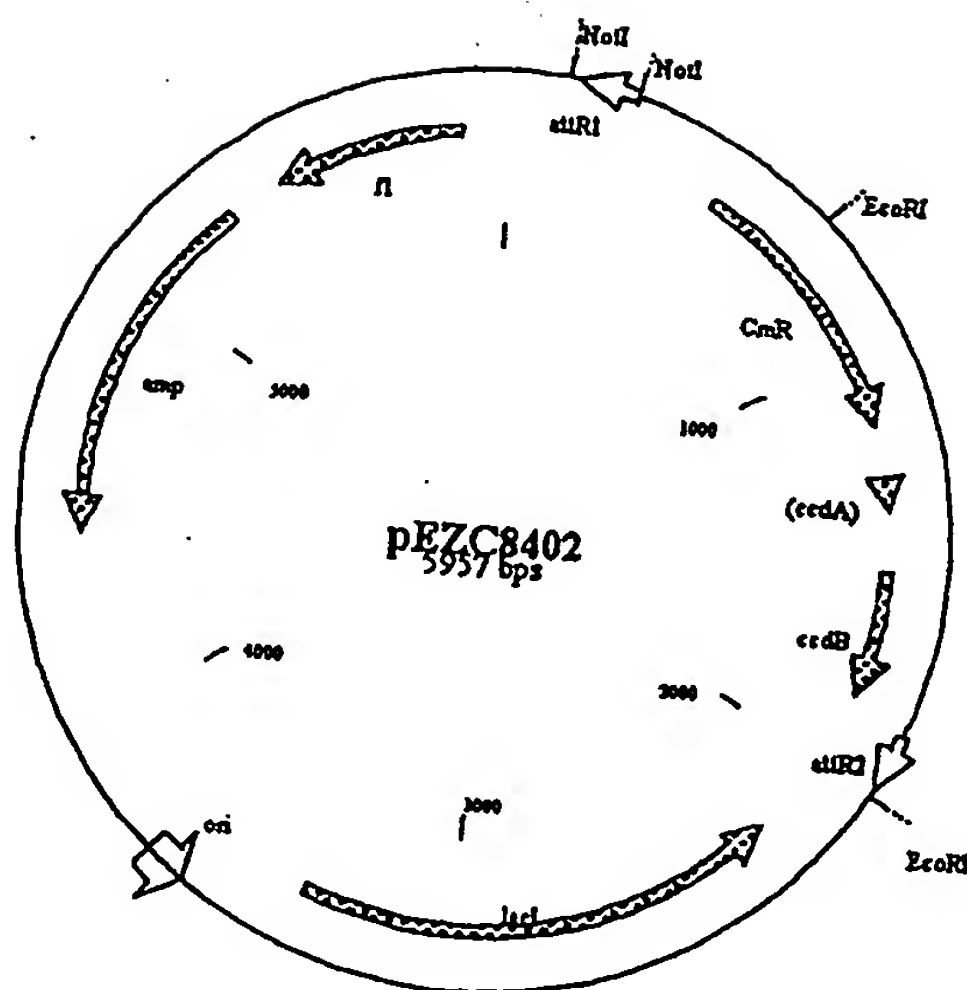


FIGURE 5B

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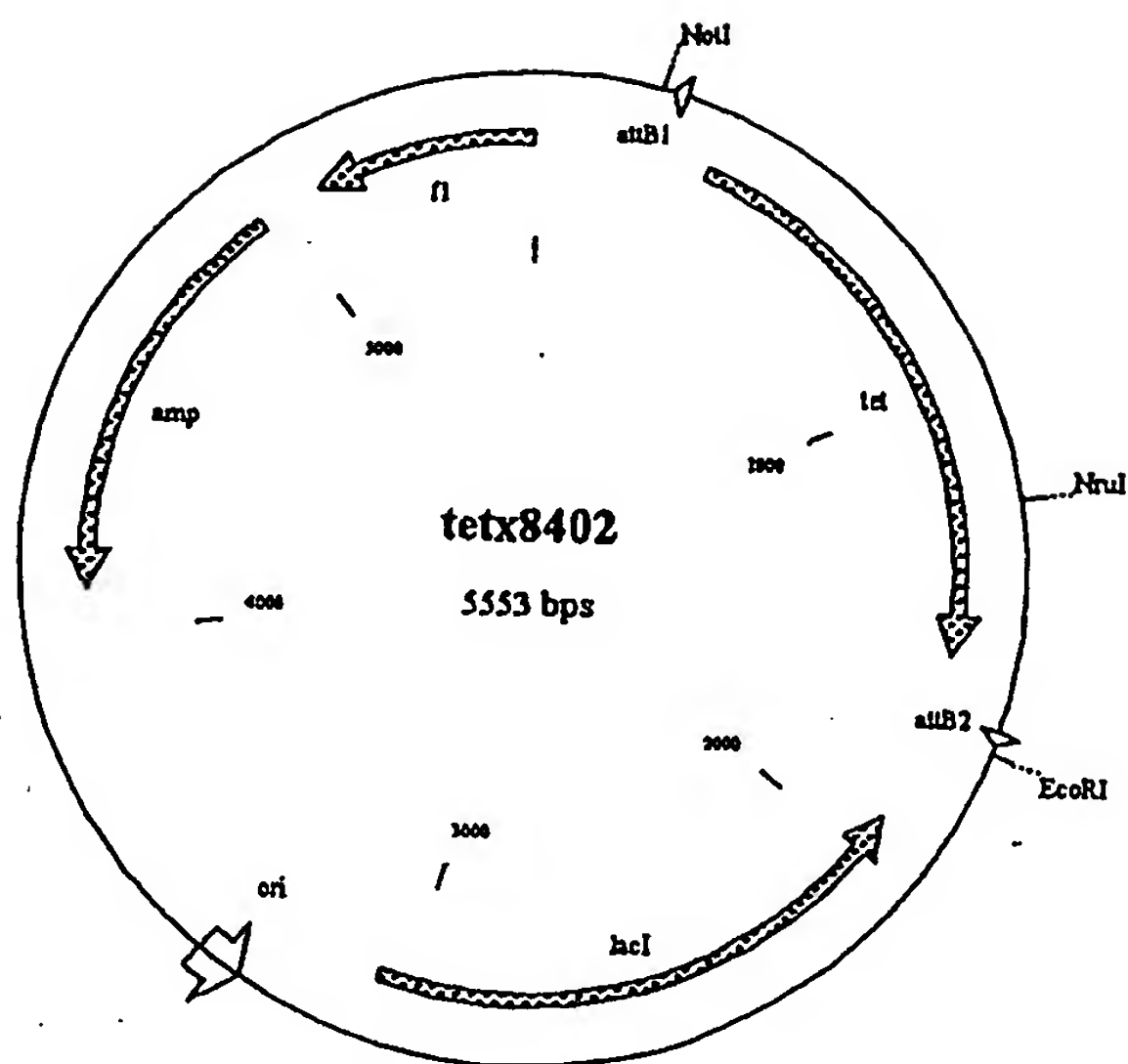


FIGURE 59

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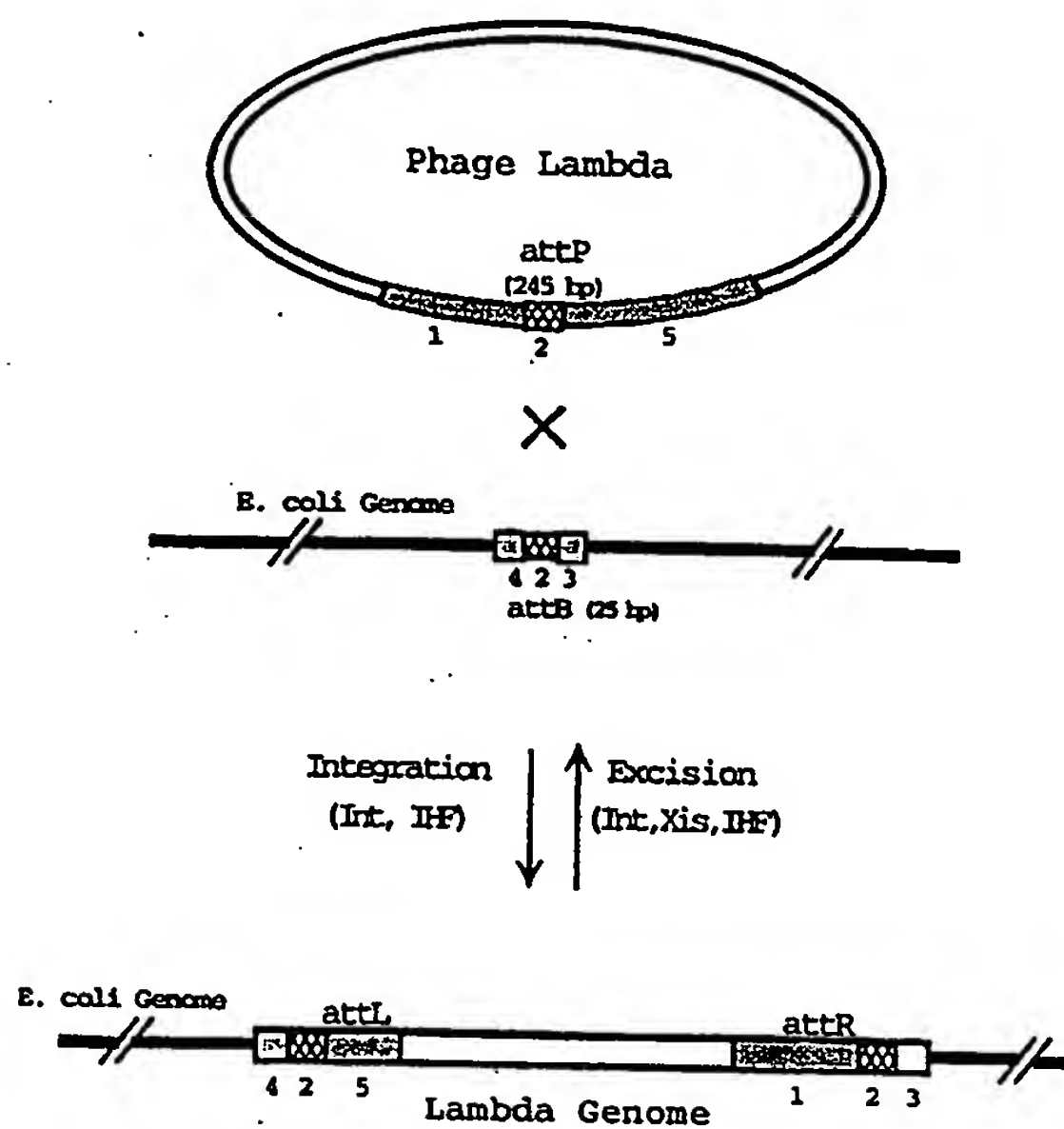


FIGURE 60

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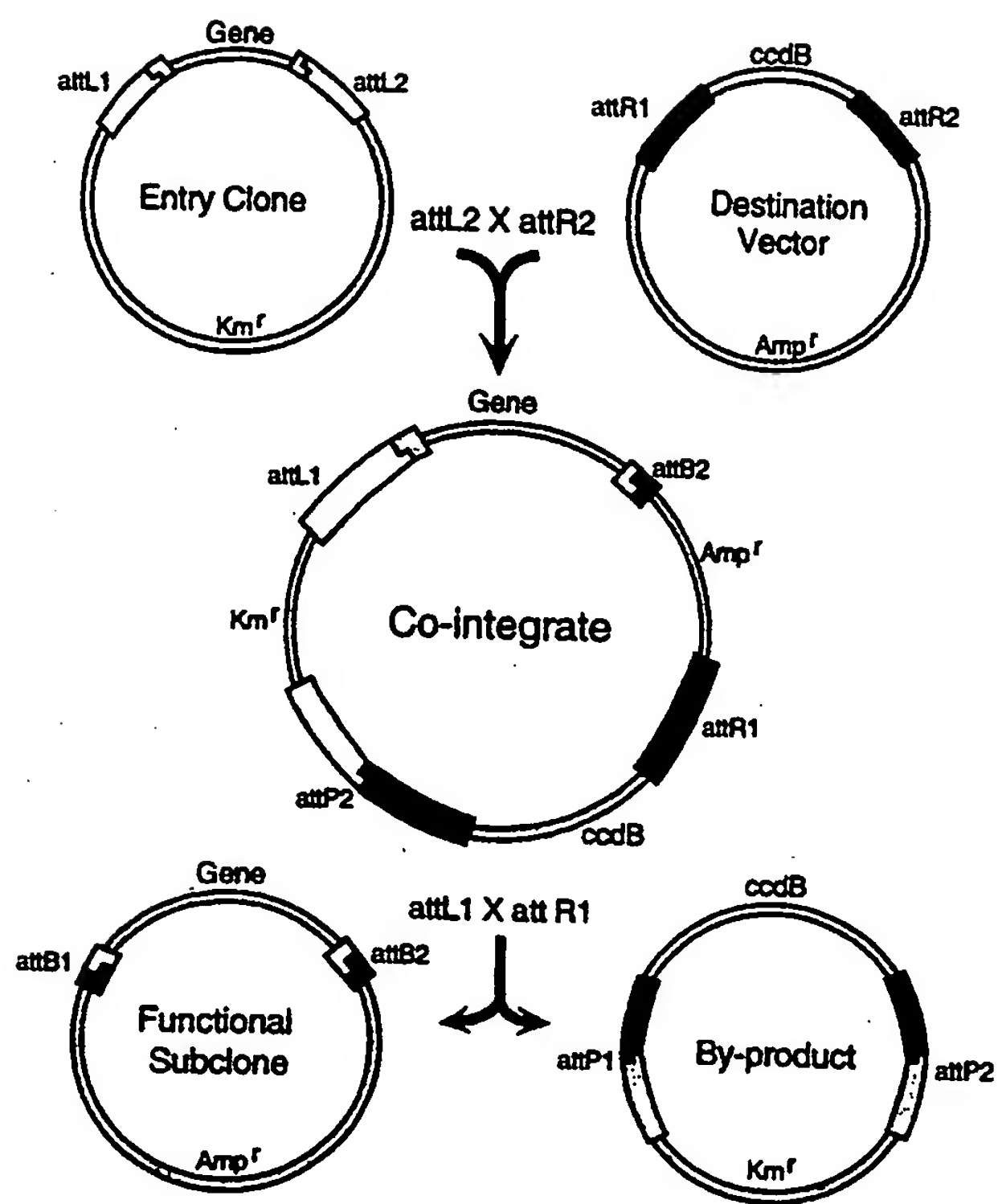


FIGURE 61

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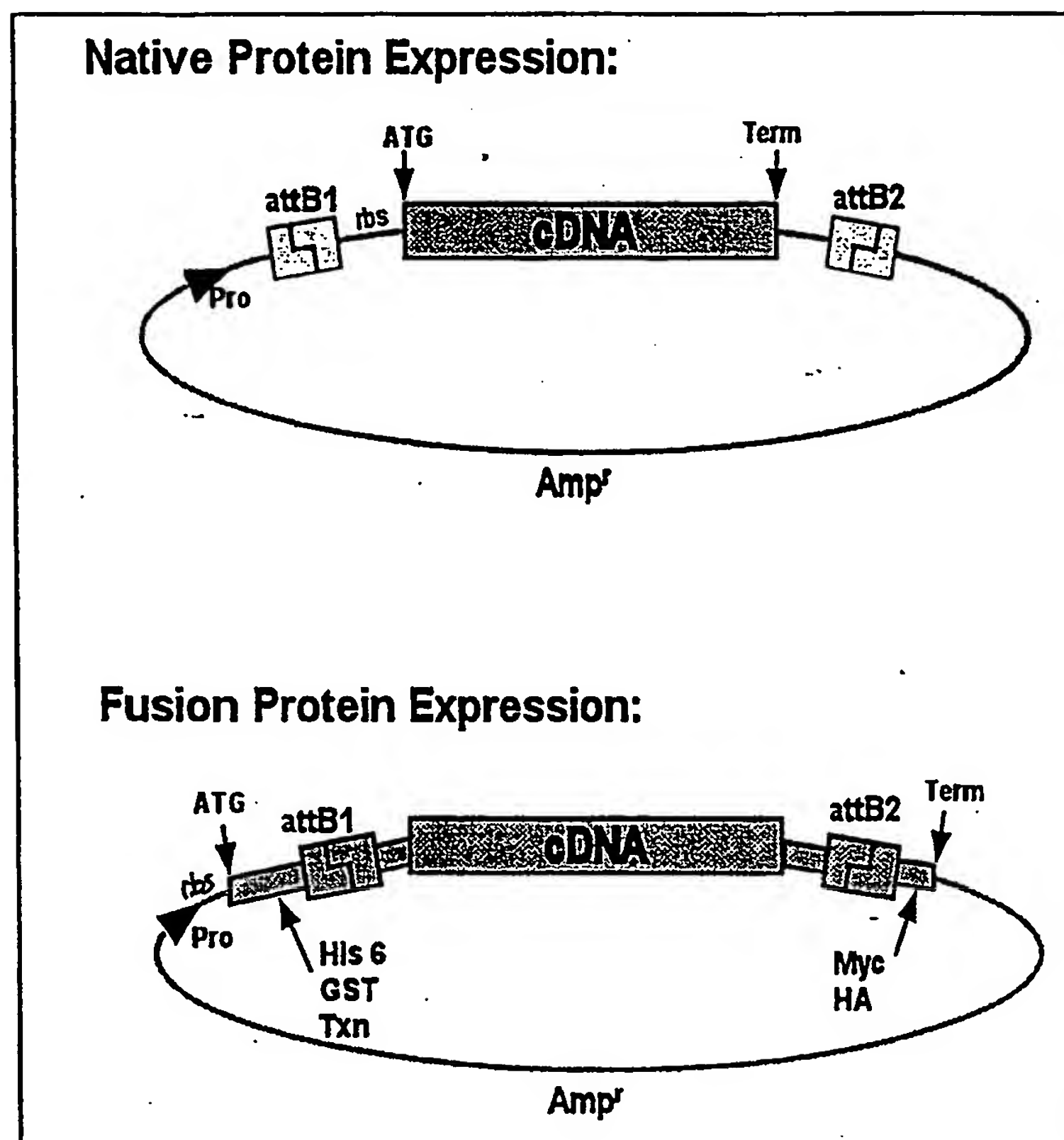


FIGURE 62



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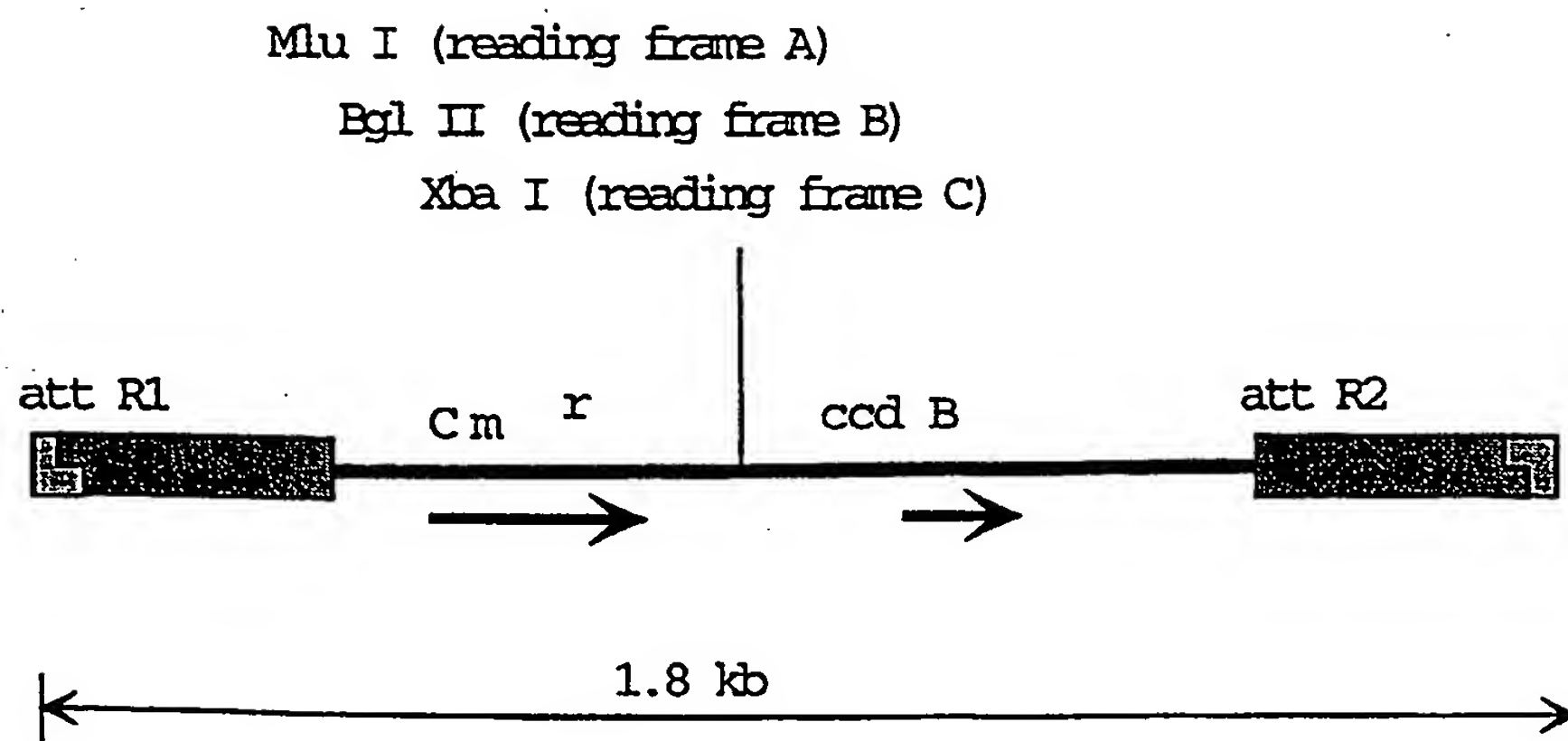
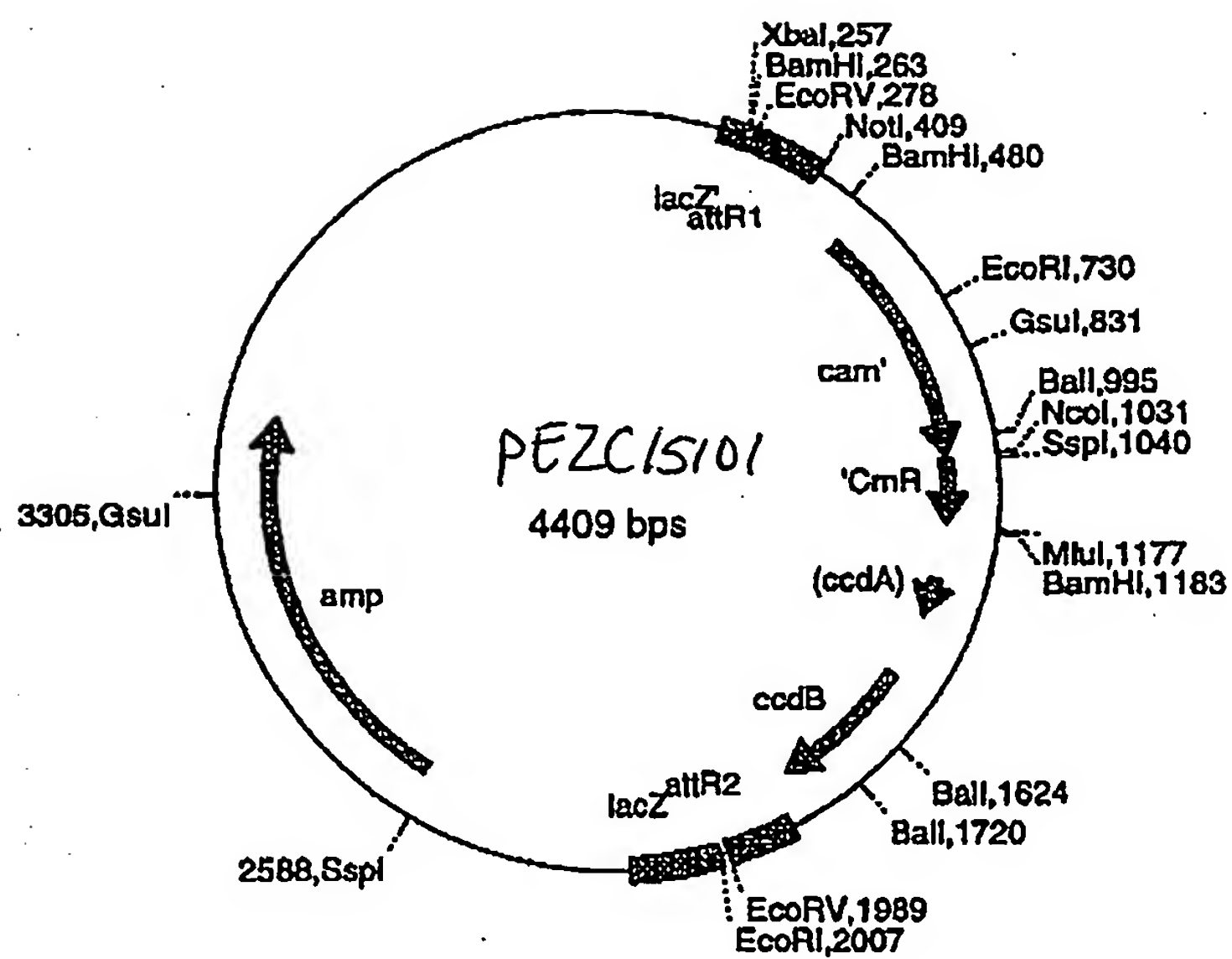


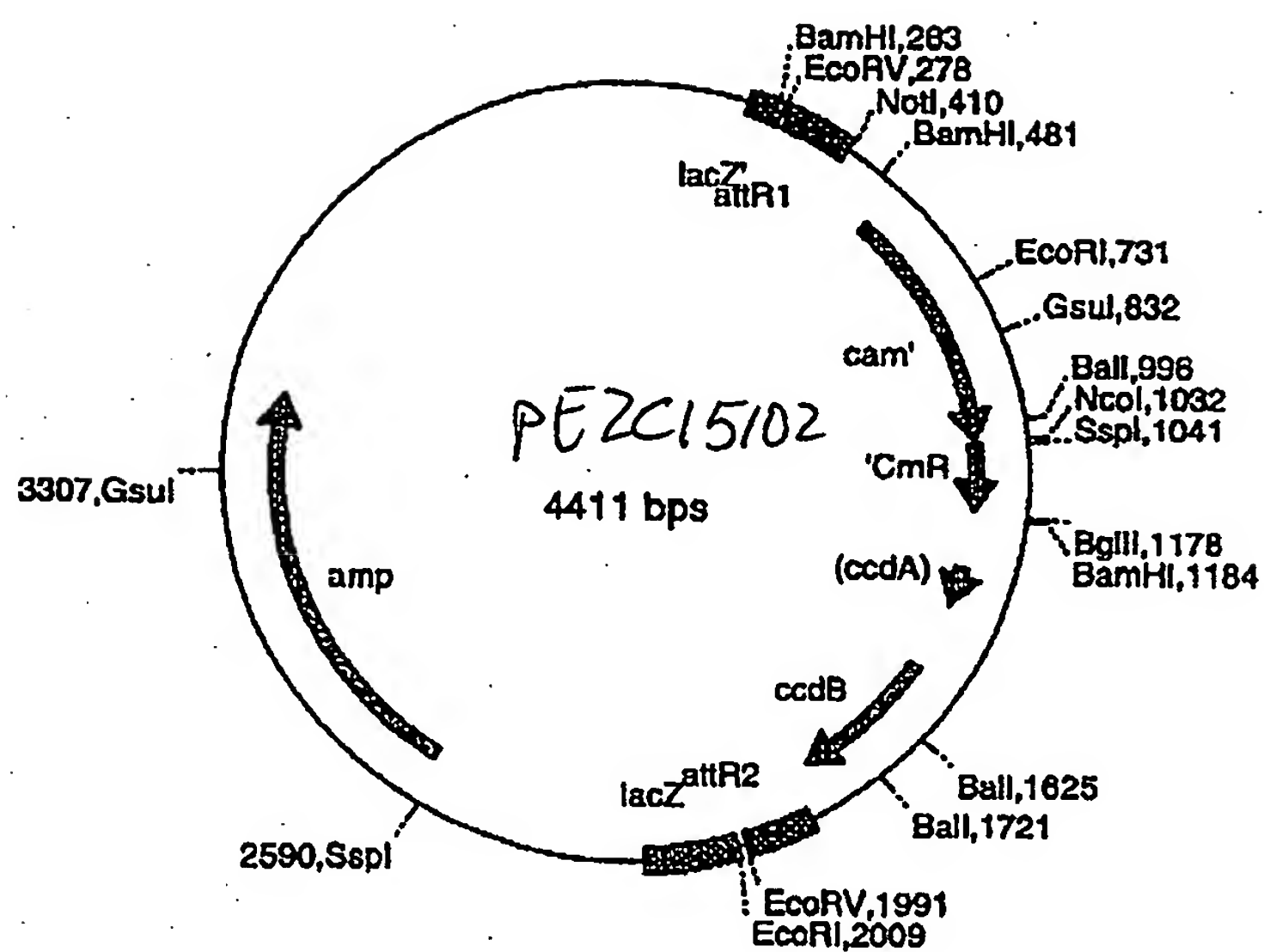
FIGURE 63

FIGURE 64A



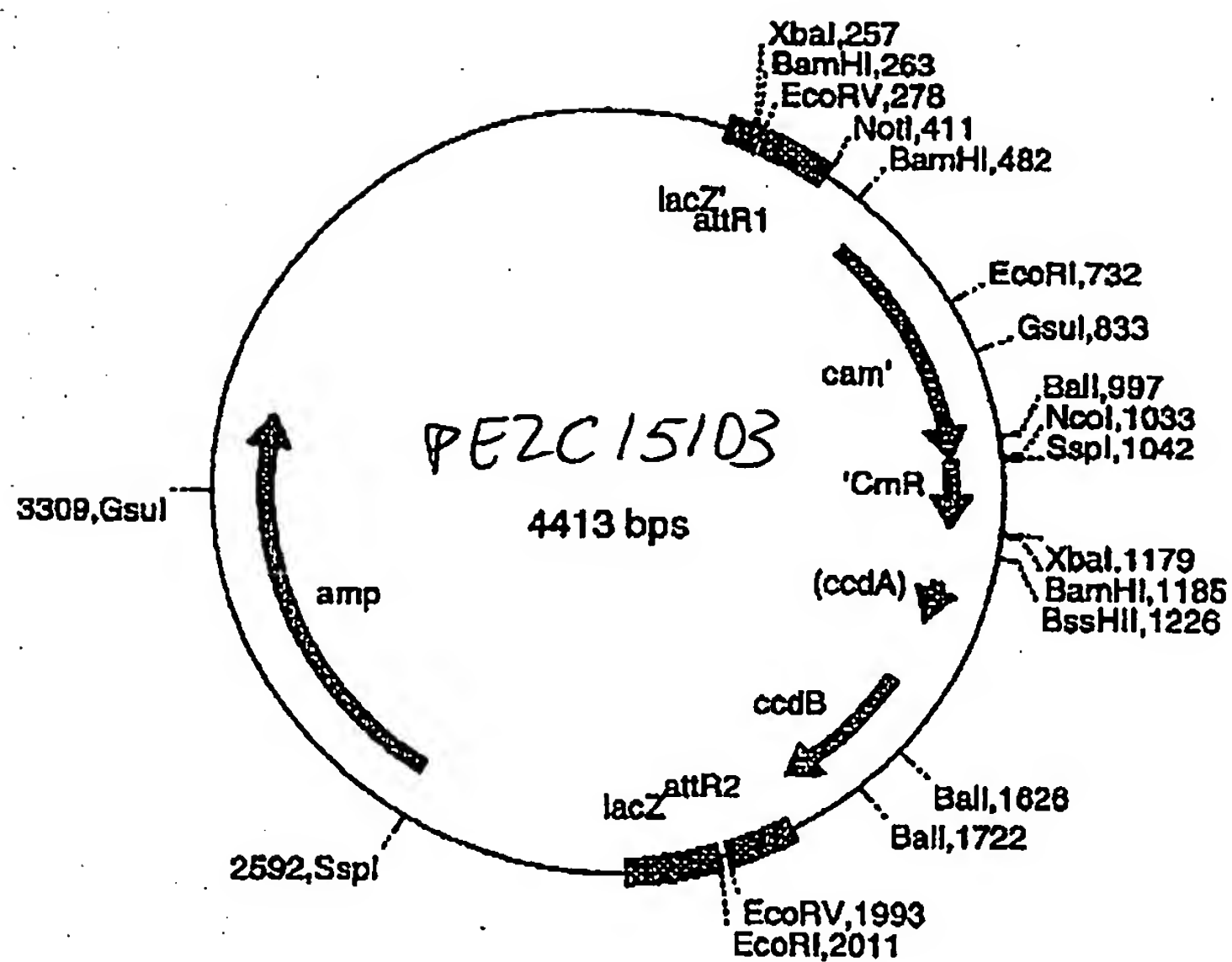
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FIGURE 4A



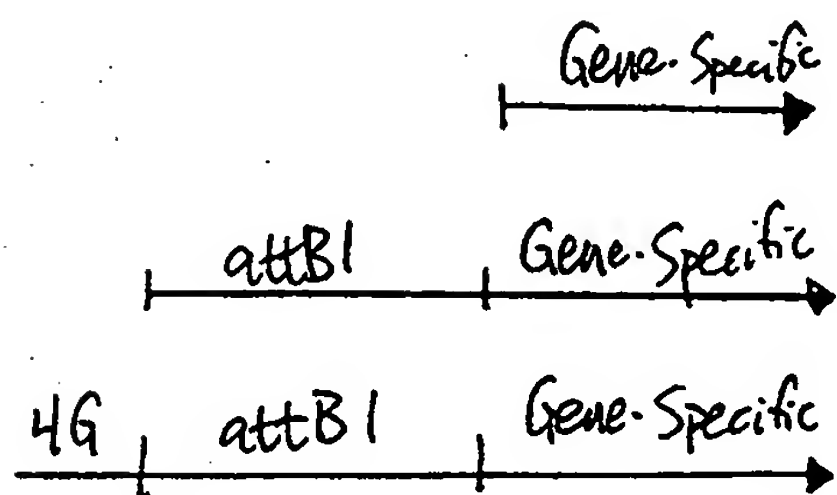
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FIGURE 64C



# Primers for Amplifying tetR and ampR for Cloning by Recombination

## Primers



## Reverse Primers

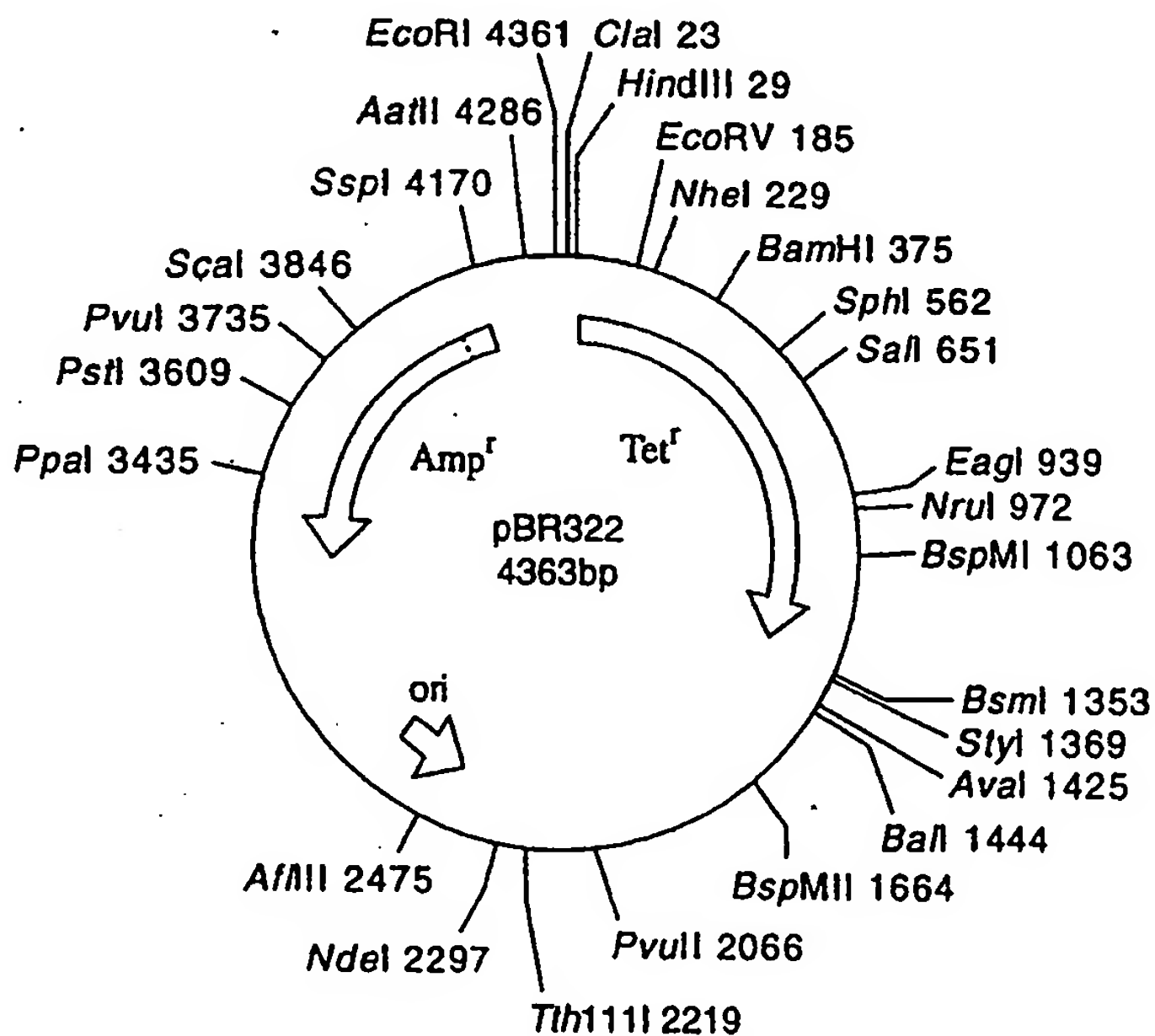
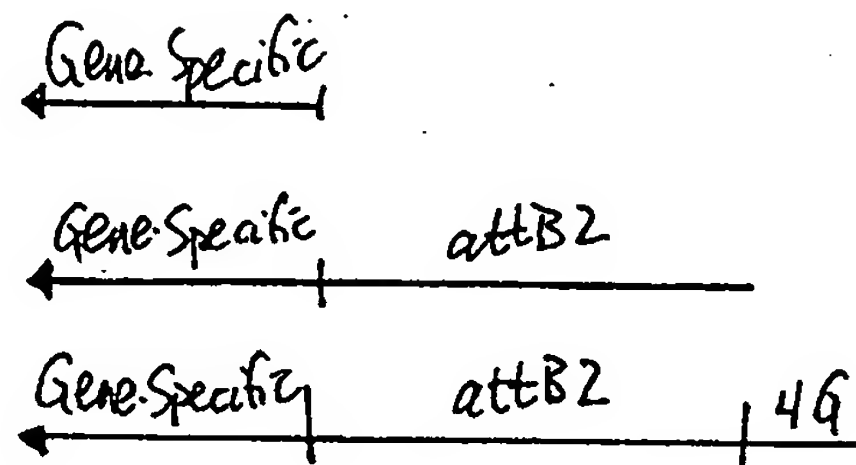


FIGURE 05

**Results of Cloning  
tet and amp PCR Products  
by Recombination**

<b>PCR Product Used in GCS Reactions</b>	<b>No. Colonies Obtained (100 ul plated)</b>	<b>Form of DNA Analyzed</b>	<b>Colonies Obtained of Predicted Size</b>
<b>tet</b>	<b>6, 10</b>	<b>SC</b>	<b>0 of 8</b>
<b>attB-tet</b>	<b>9, 6</b>	<b>SC</b>	<b>1 of 8</b>
<b>attB+4G-tet</b>	<b>824, 1064</b>	<b>SC AvaI+Bam</b>	<b>7 of 7 7 of 7</b>
<b>amp</b>	<b>7, 13</b>	<b>SC</b>	<b>0 of 8</b>
<b>attB-amp</b>	<b>18, 22</b>	<b>SC</b>	<b>3 of 8</b>
<b>attB+4G-amp</b>	<b>3020, 3540</b>	<b>SC PstI</b>	<b>8 of 8 8 of 8</b>
<b>attB Plasmid (Pos. Control)</b>	<b>320, 394</b>		

FIGURE 66



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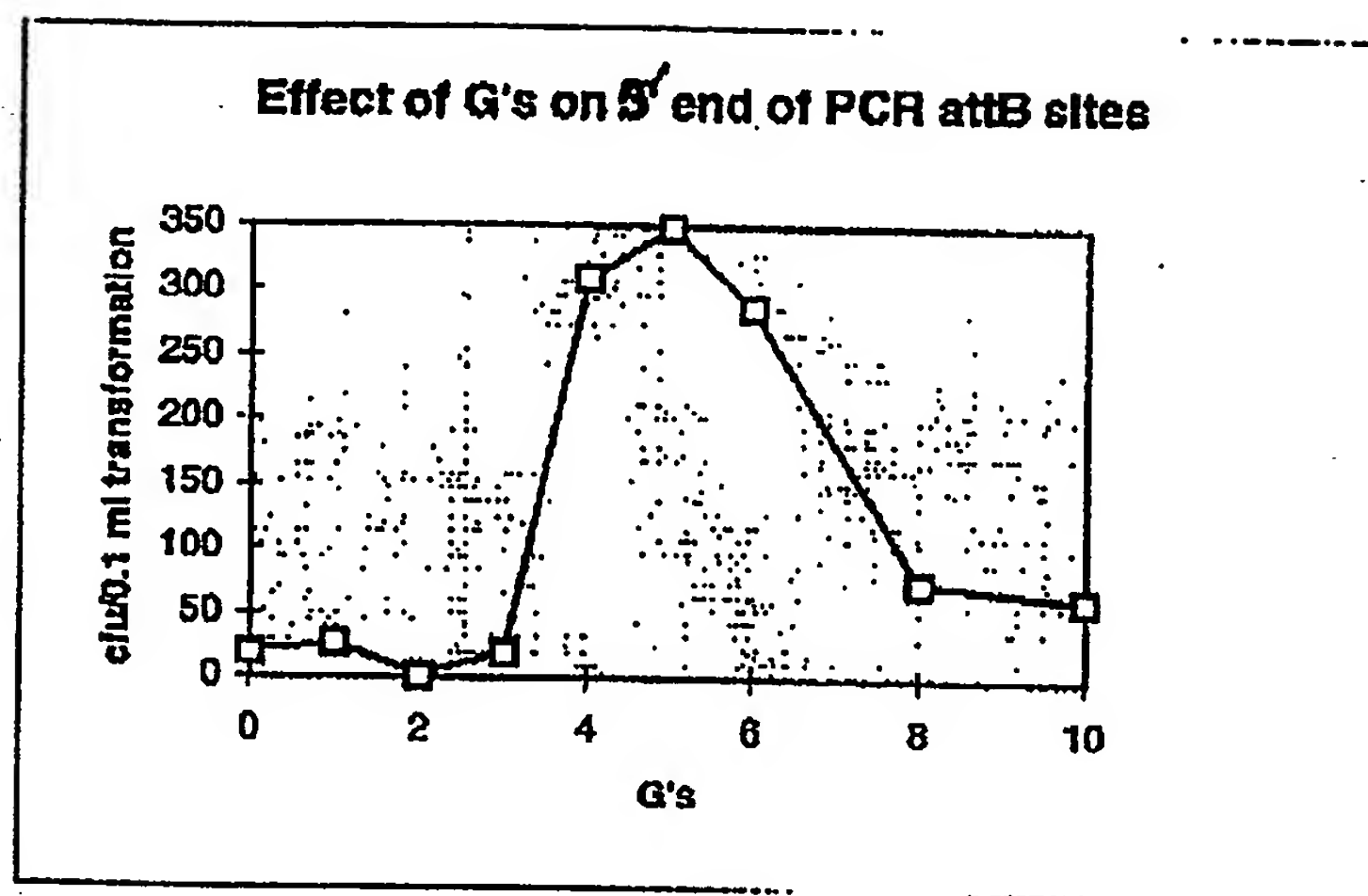


FIGURE 67

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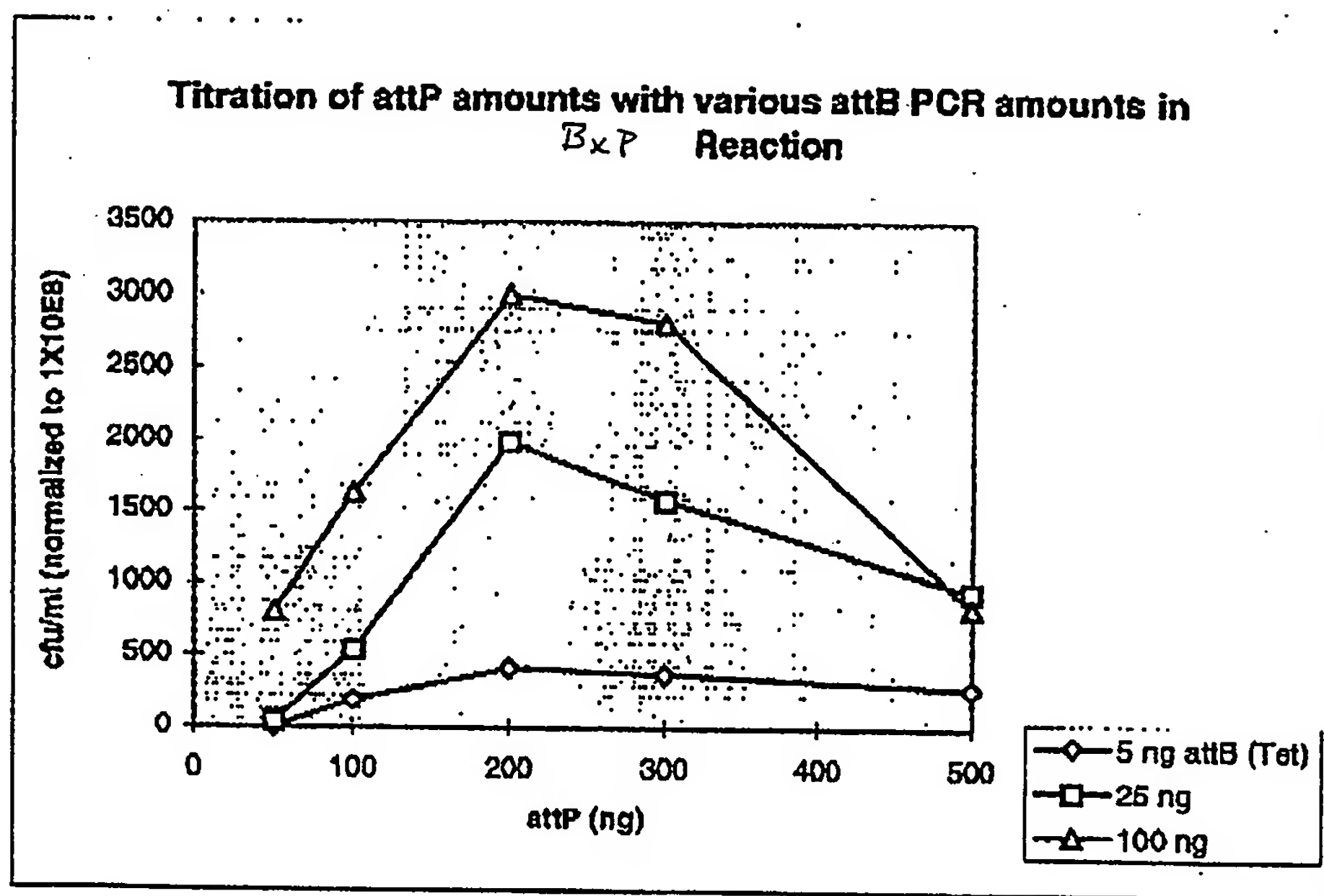
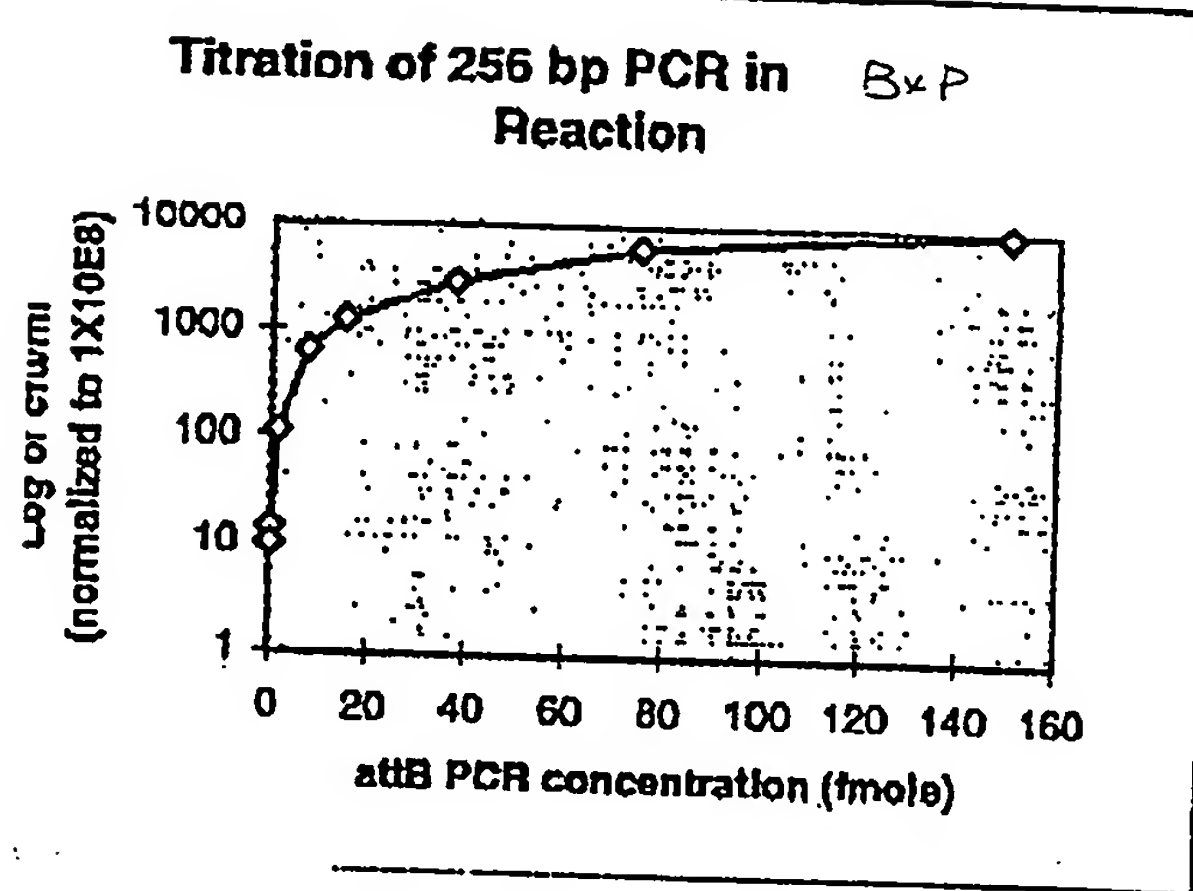


FIGURE 68

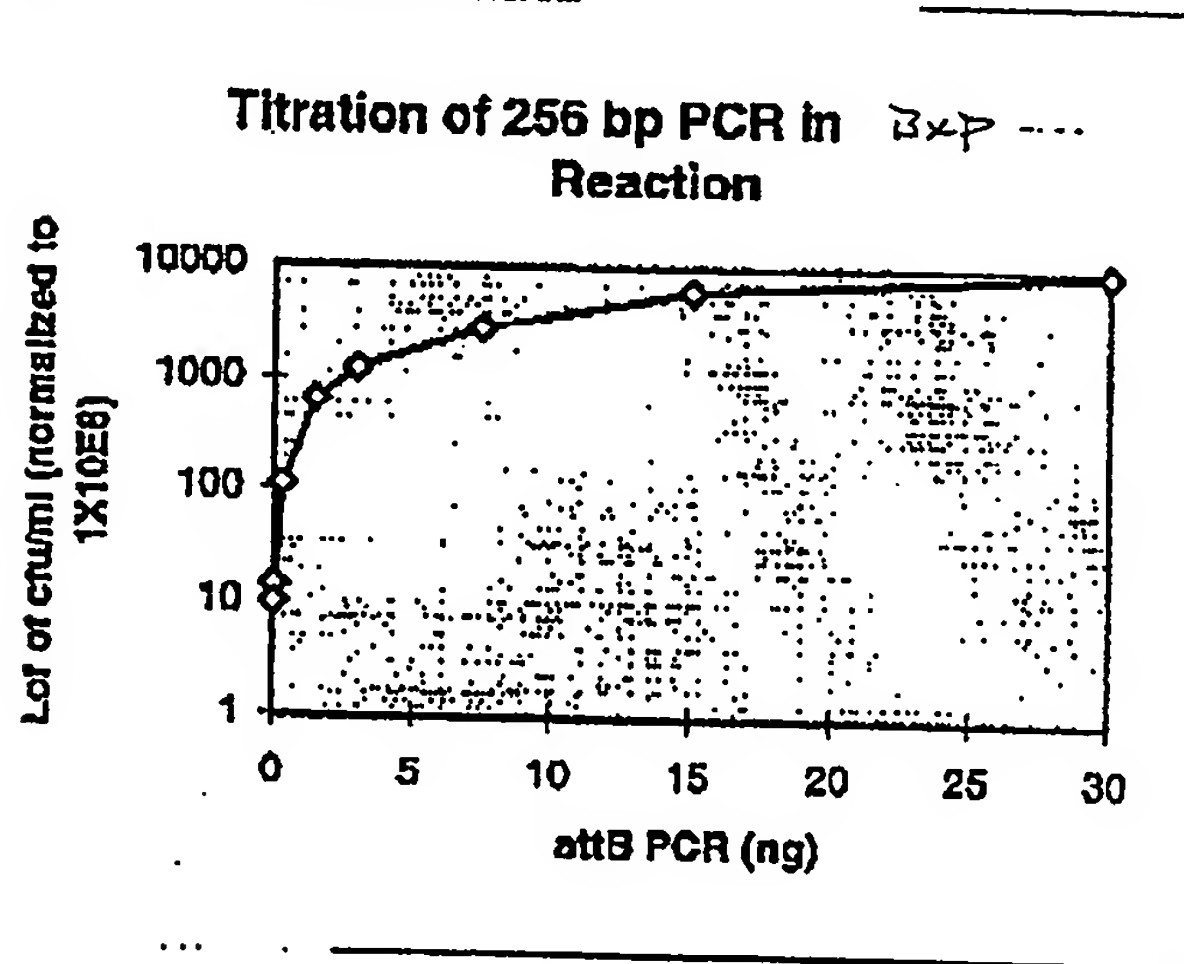
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FIGURE  
69

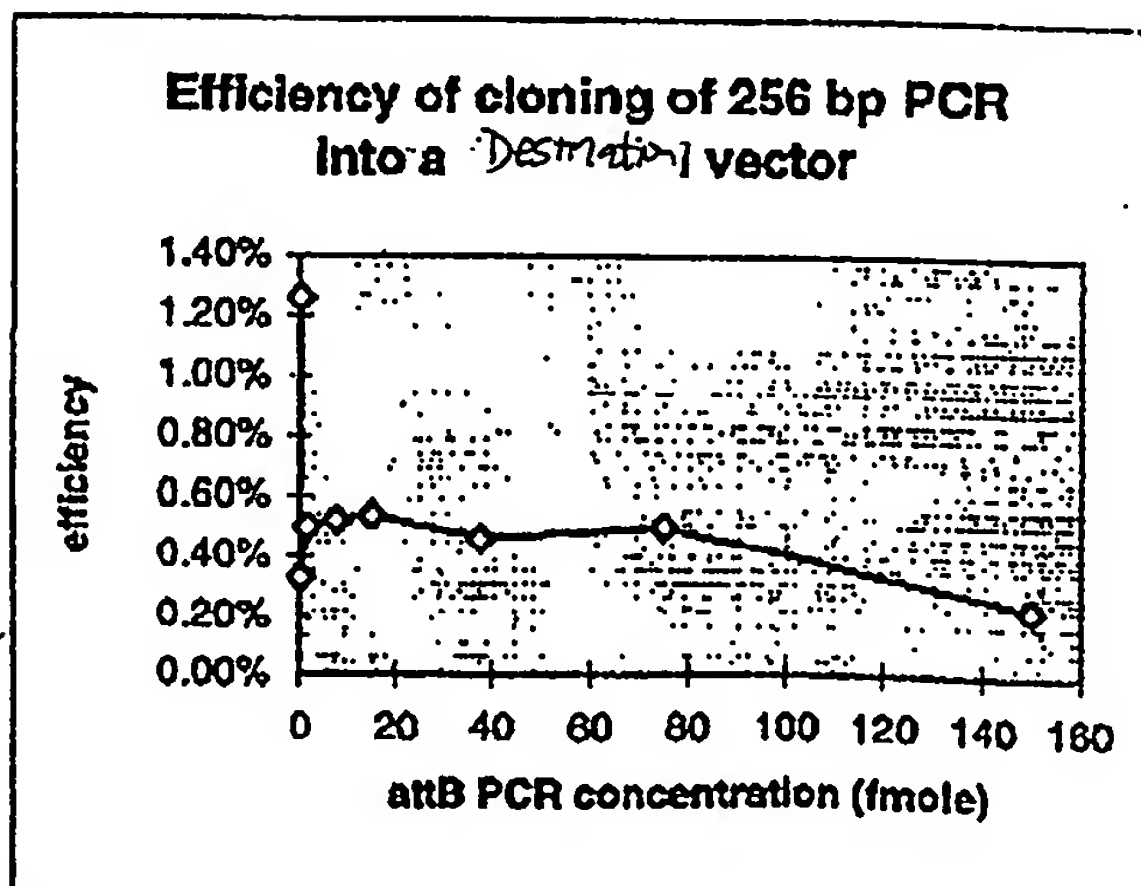
A



B



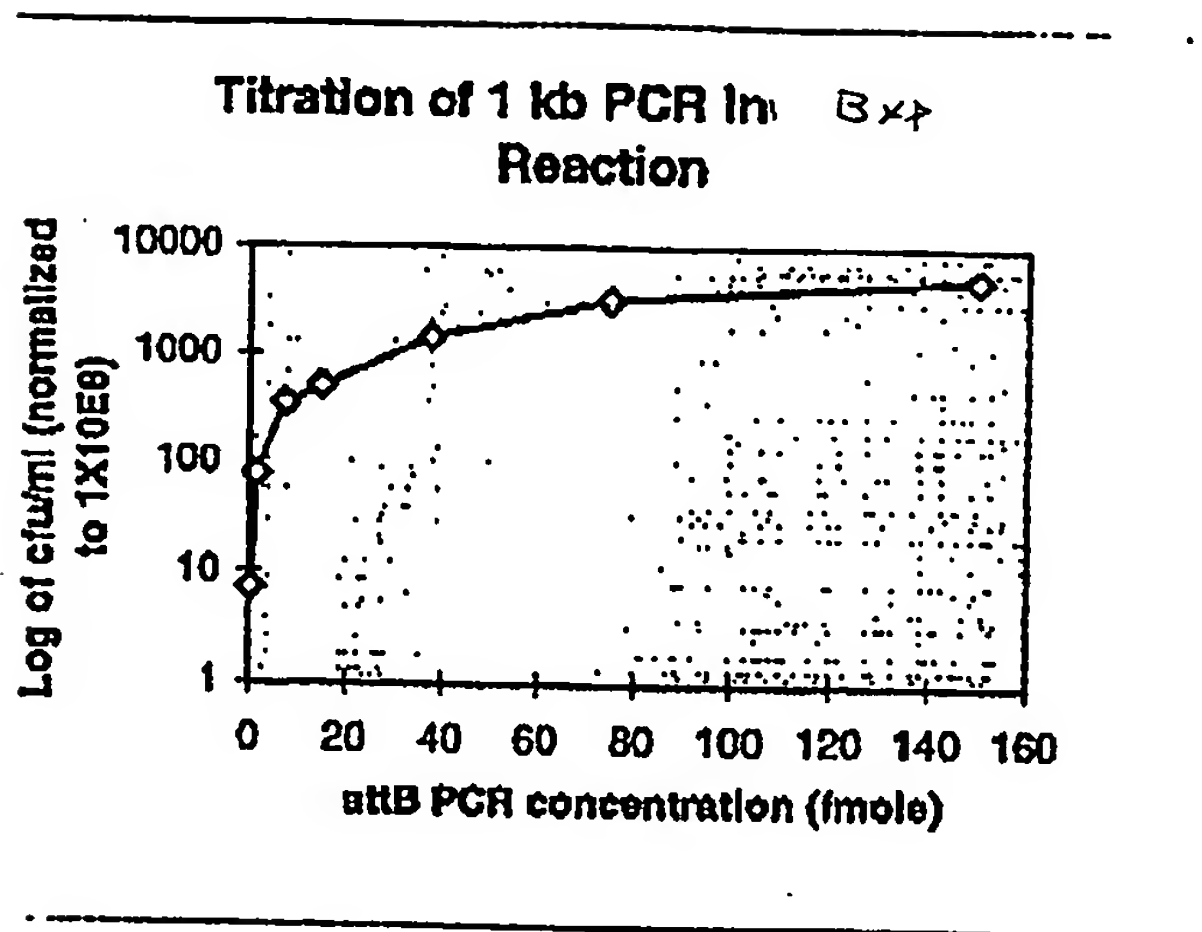
C



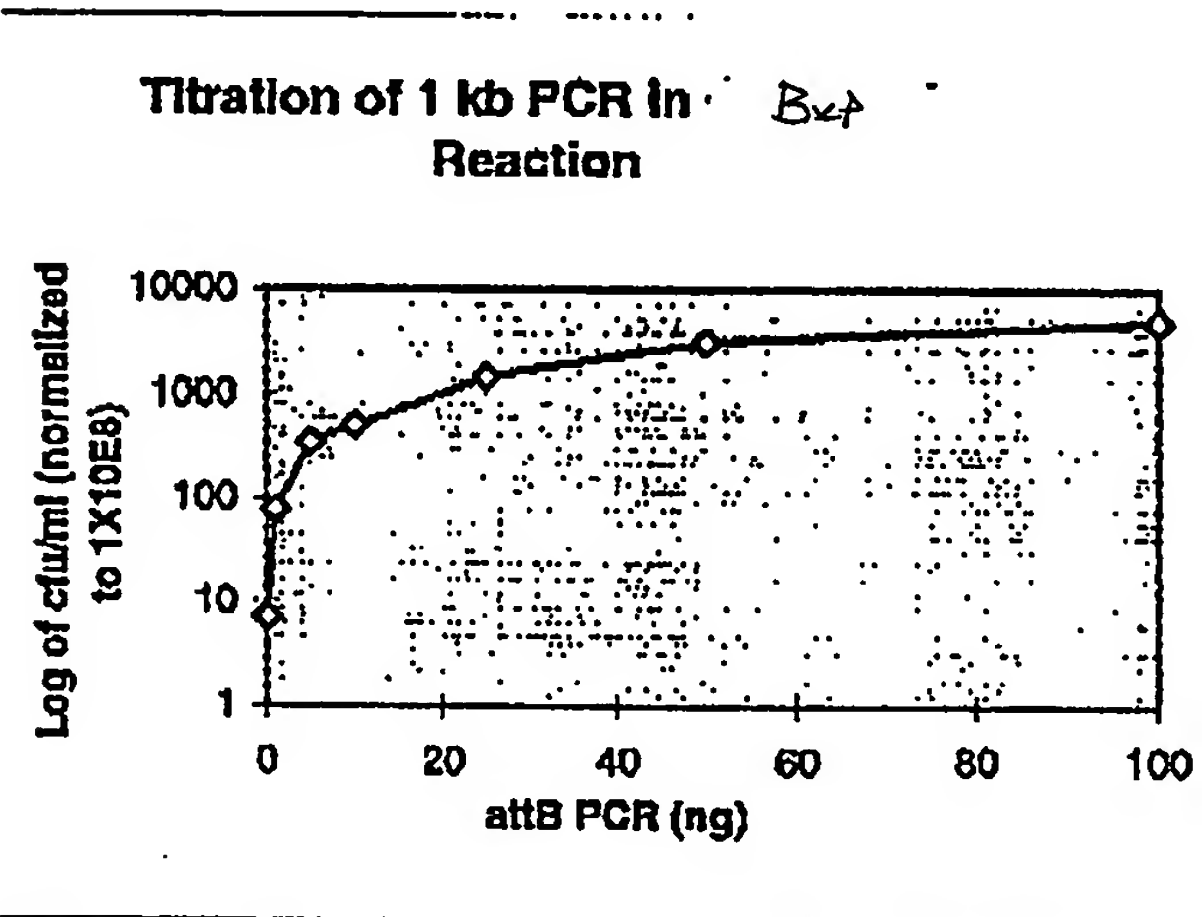
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FIGURE  
70

A



B



C

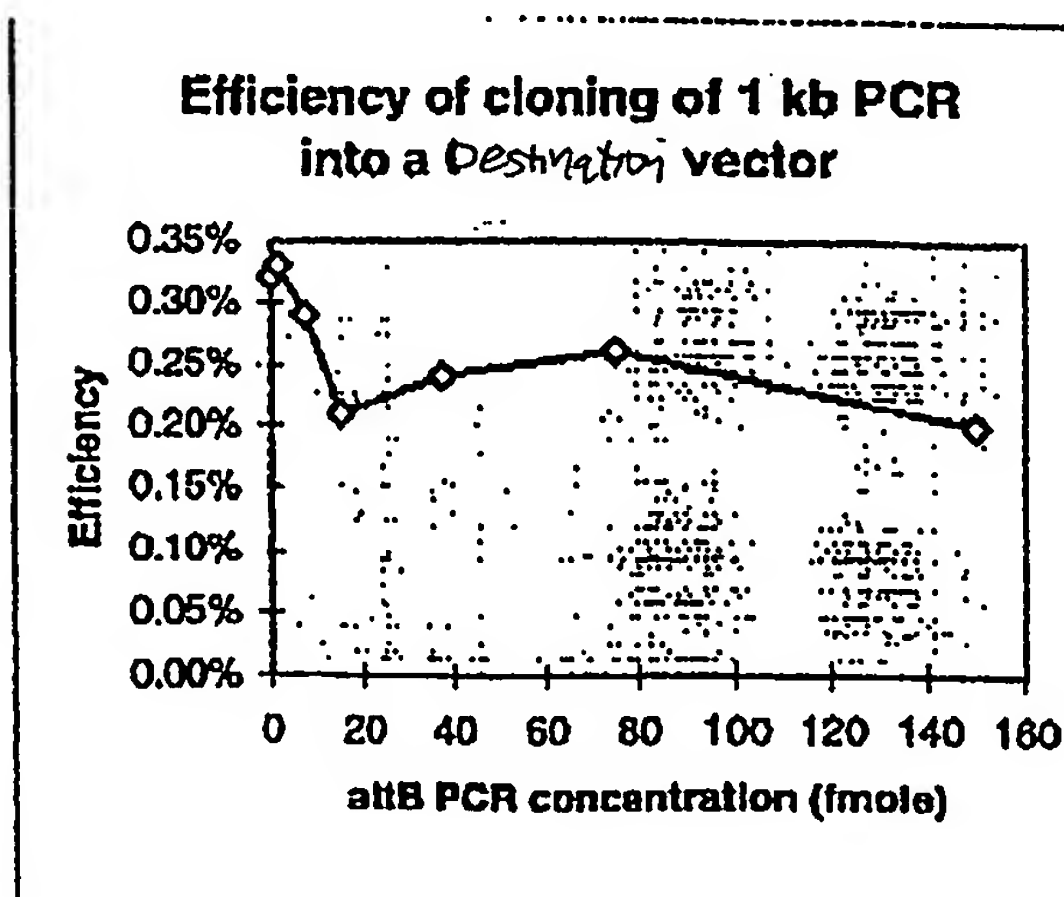
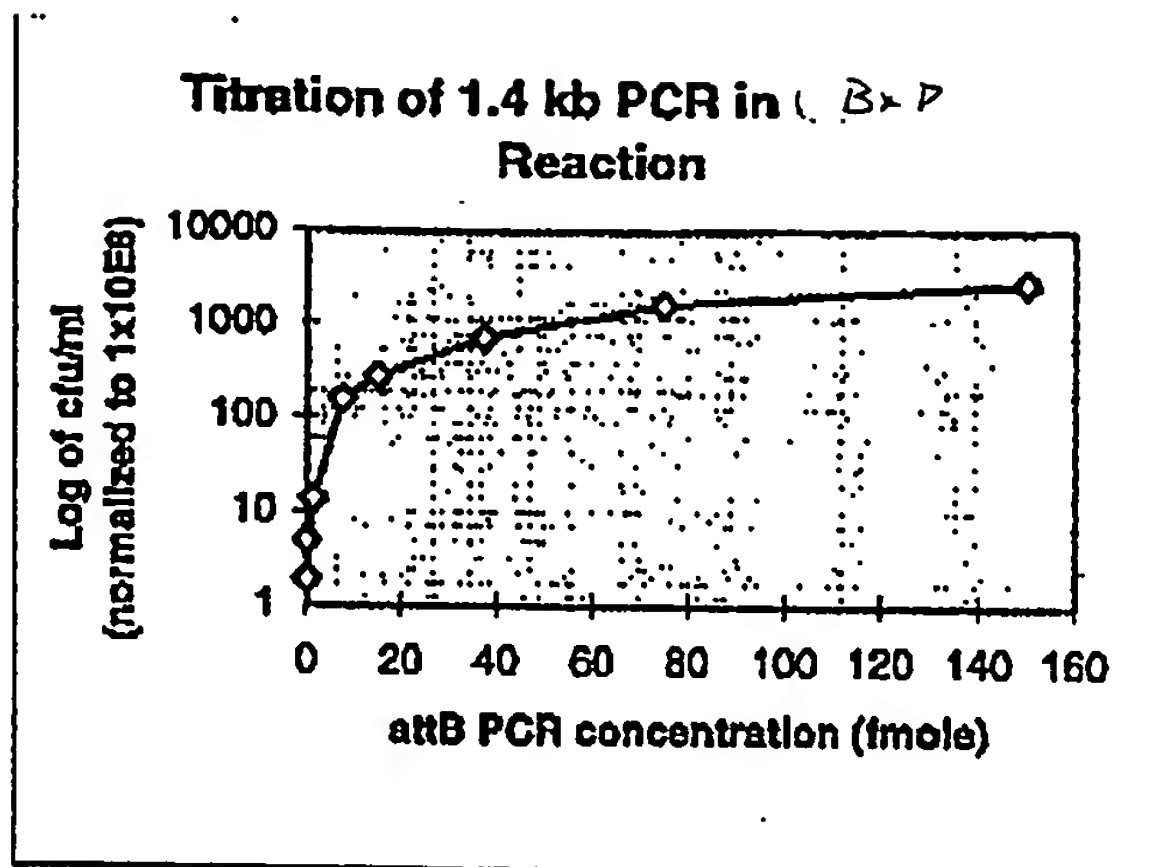
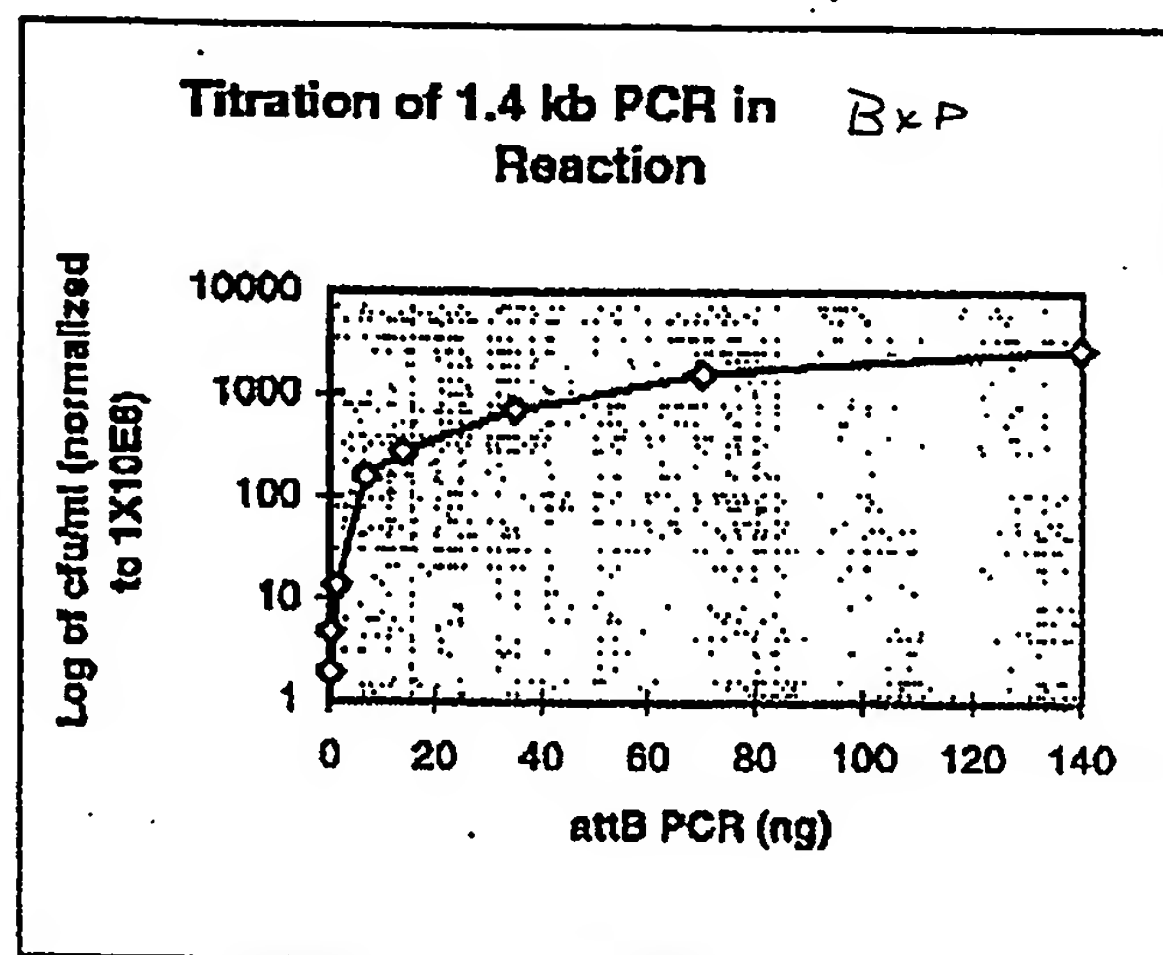


FIGURE 71

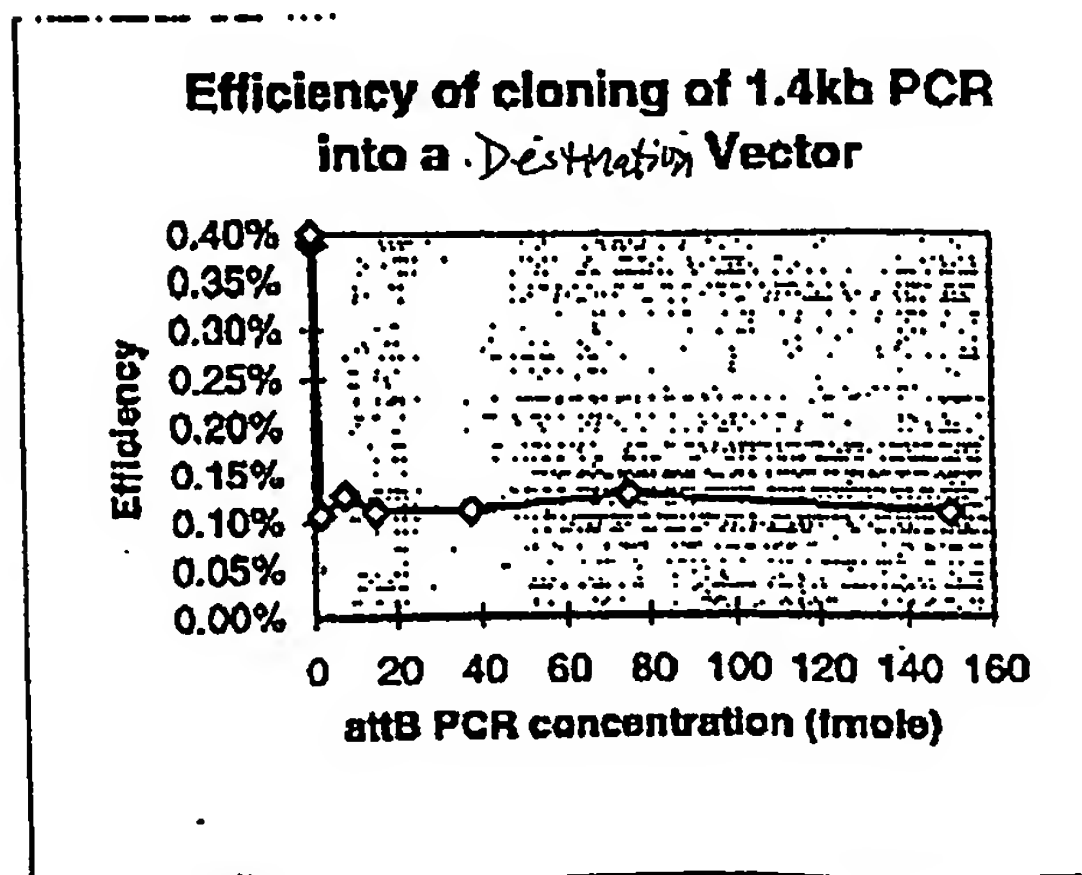
A



B



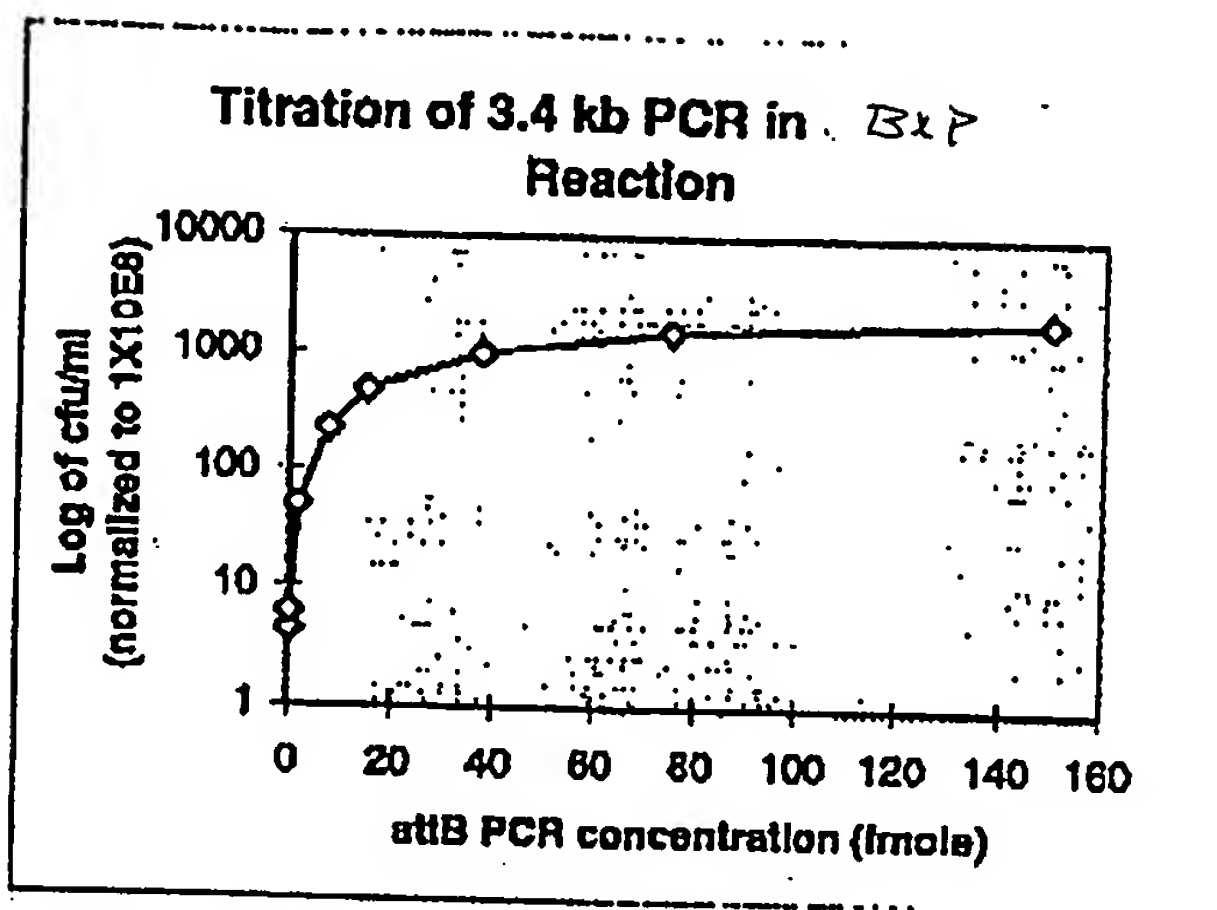
C



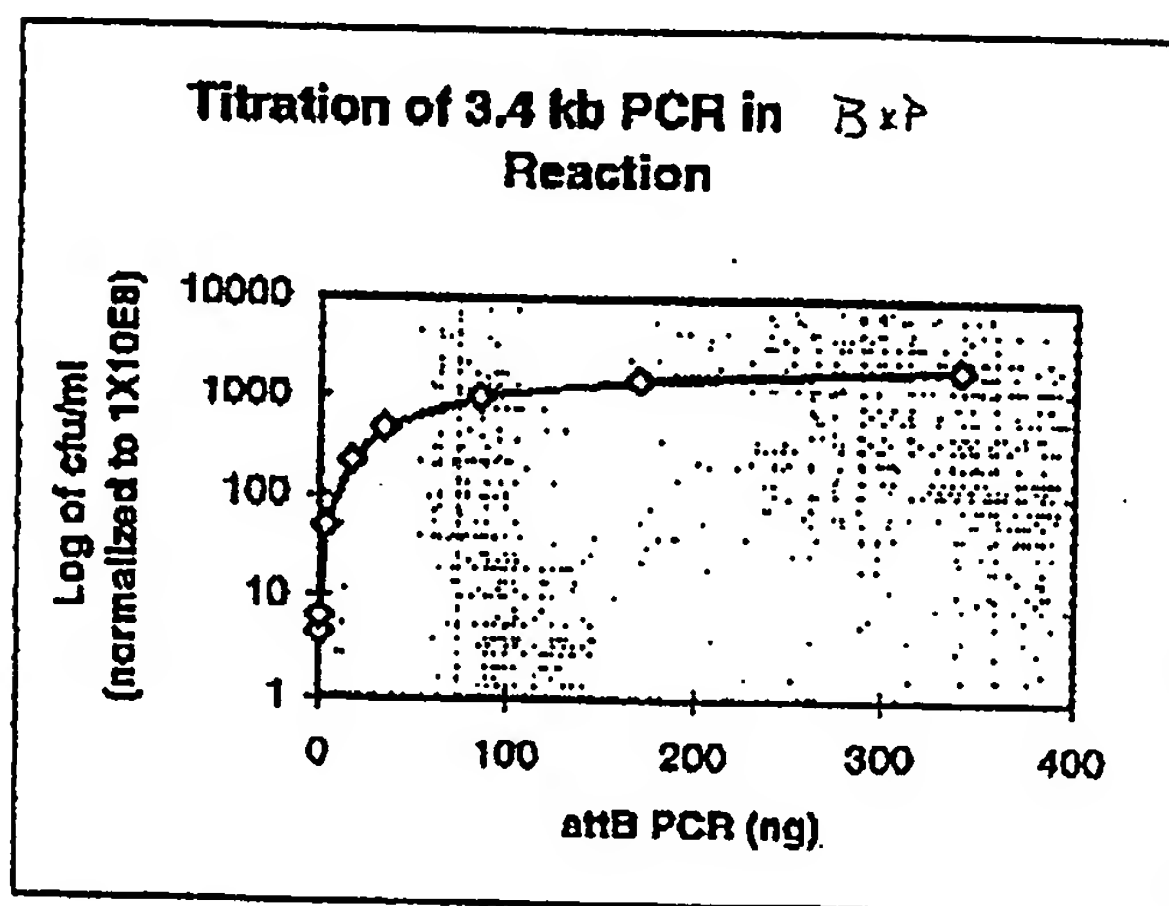
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FIGURE 72

A



B



C

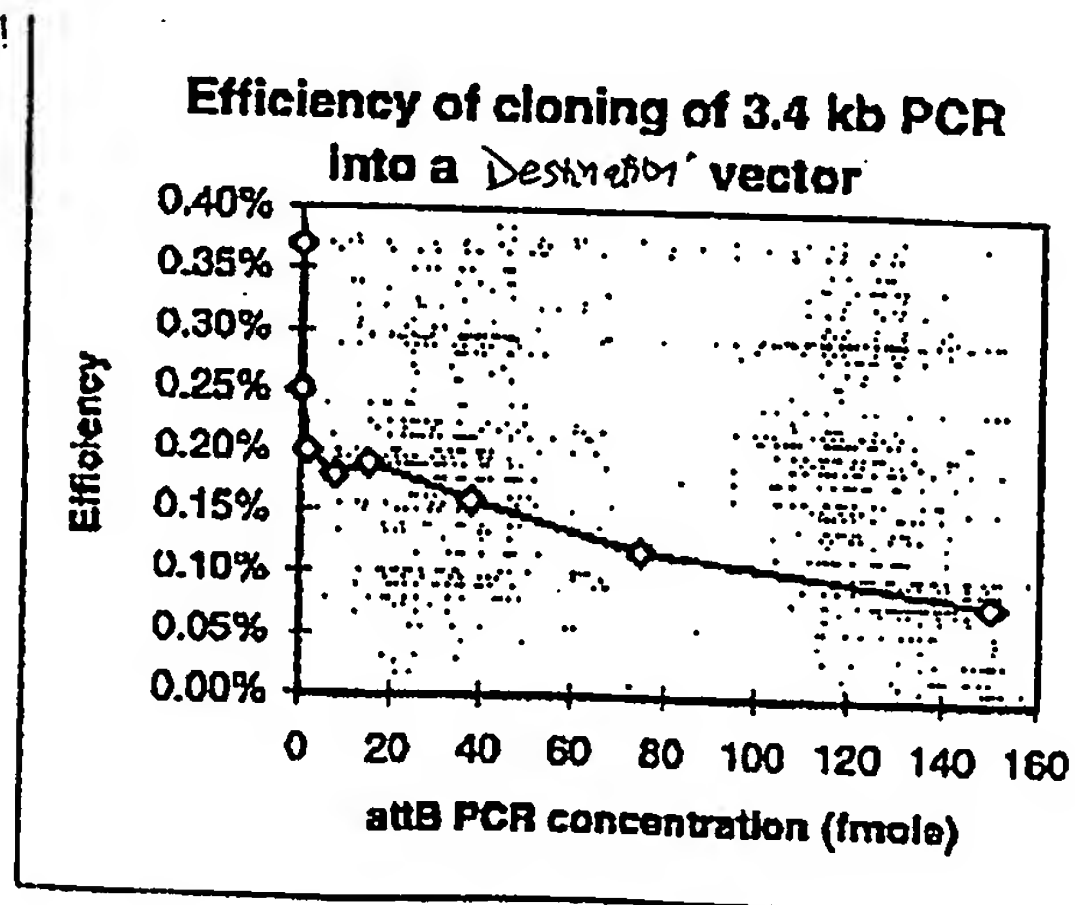
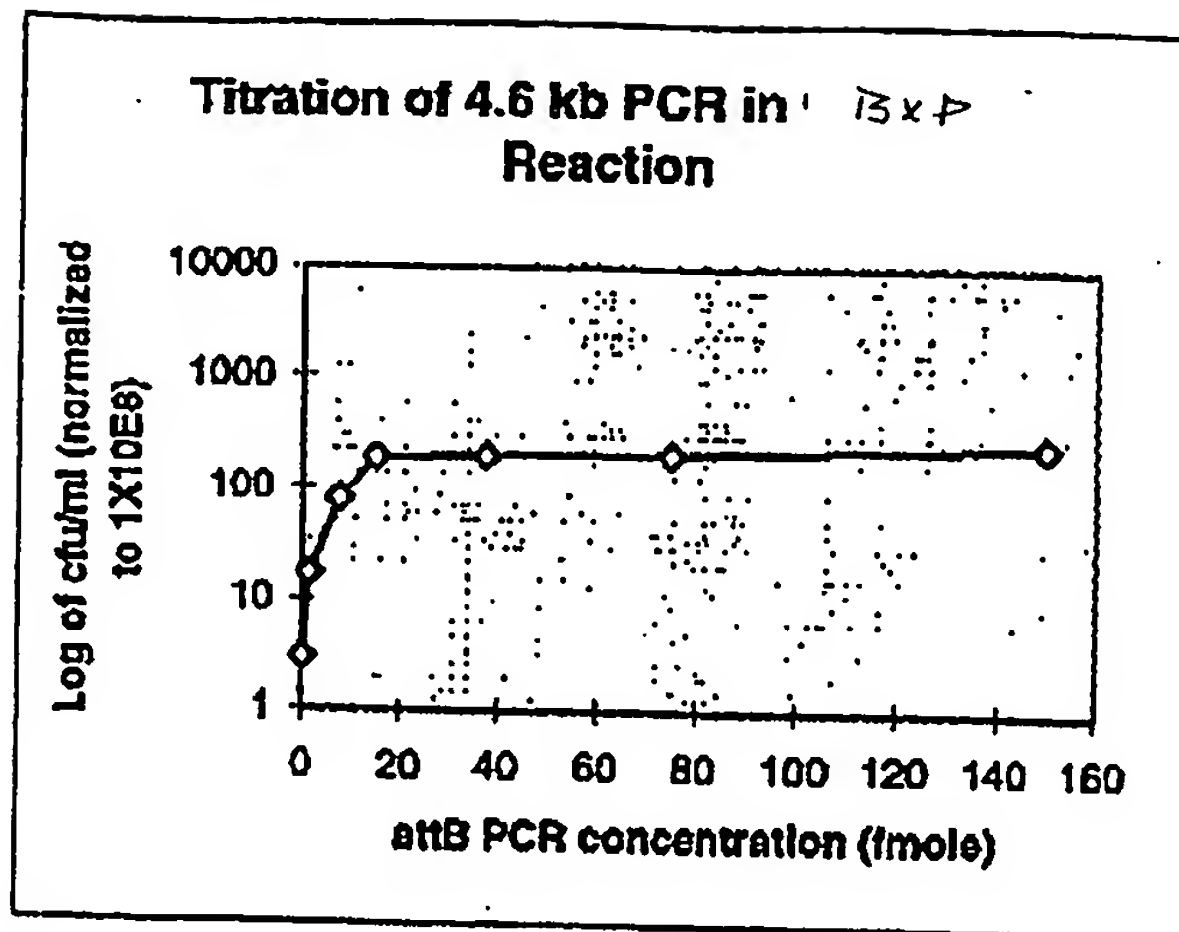


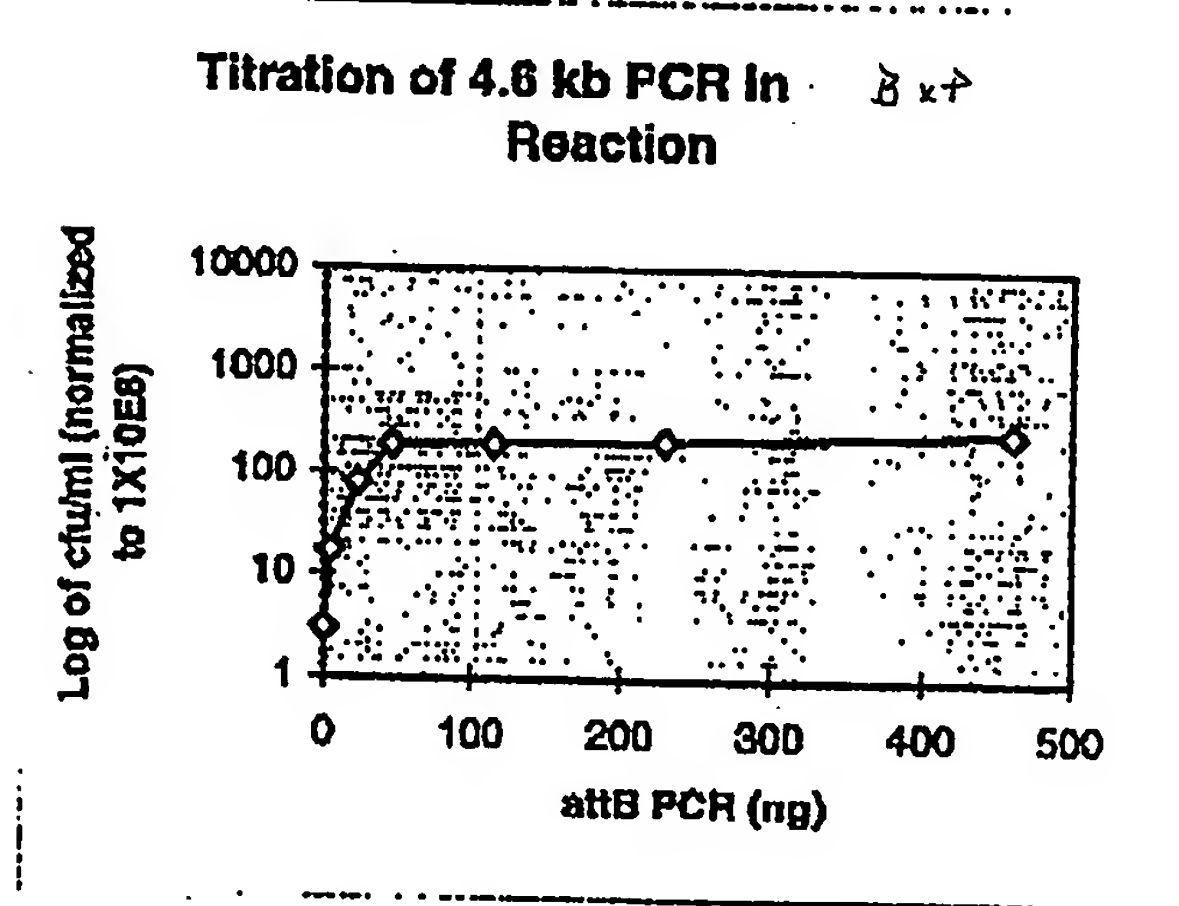


FIGURE 73

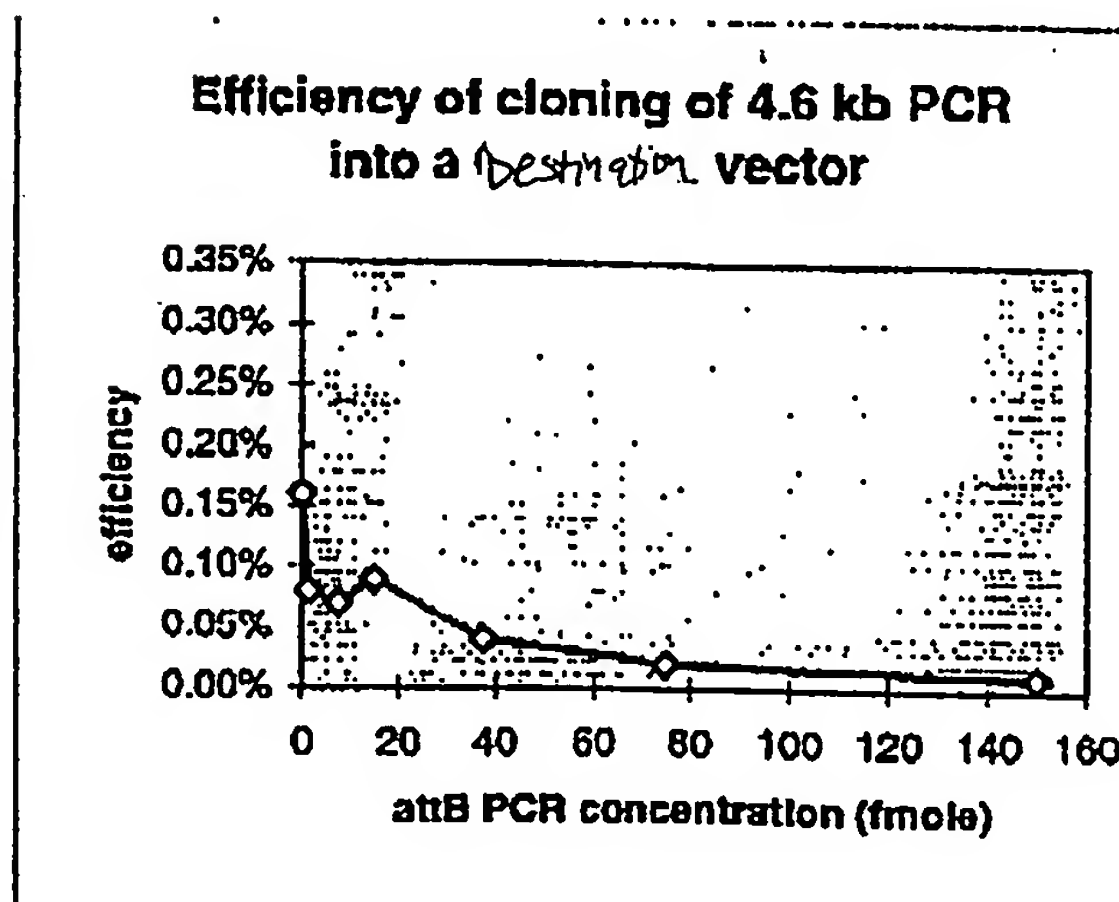
A



B



C



6.9 kb PCR DNA Titration in  $\alpha$  BxP Reaction

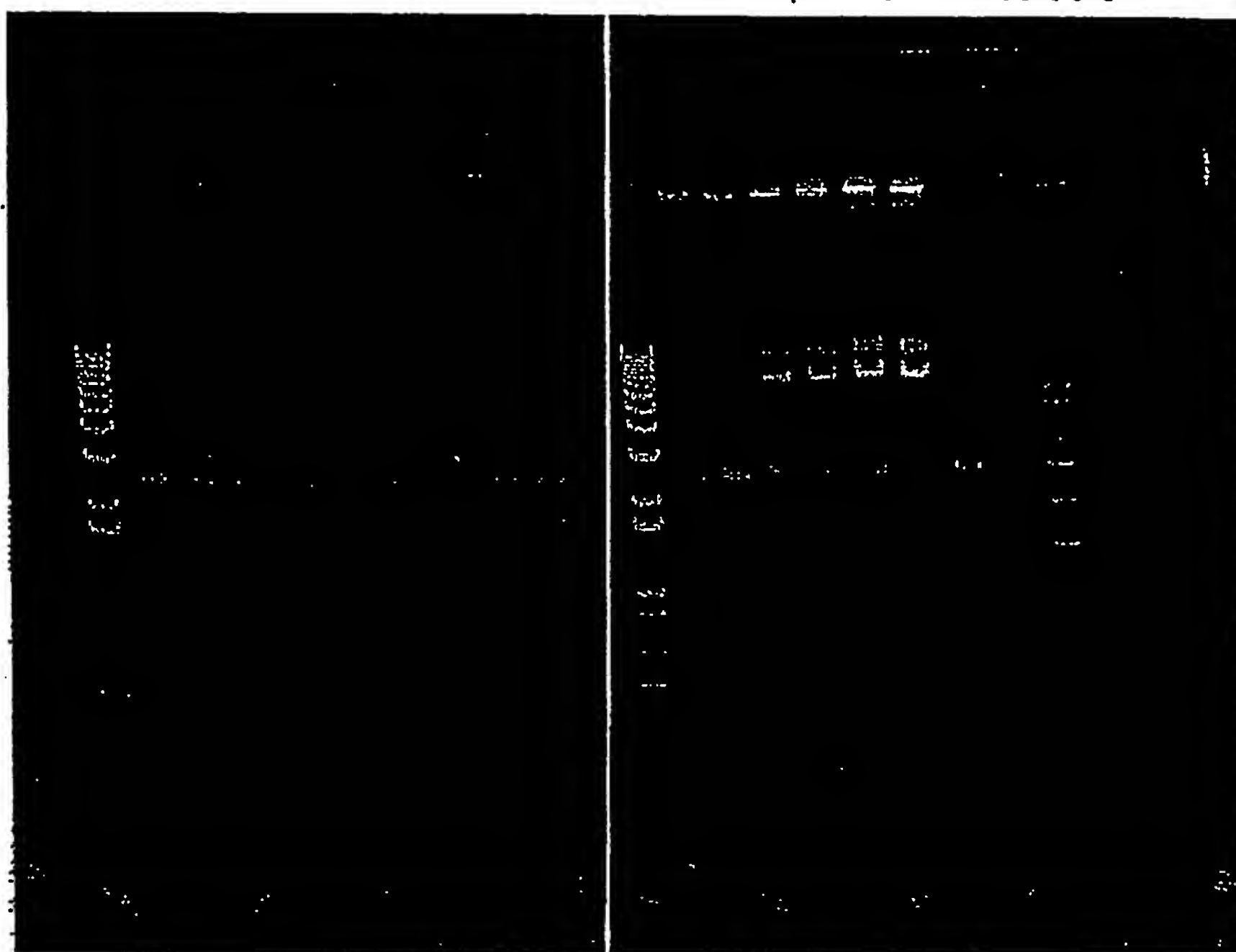


FIGURE 74

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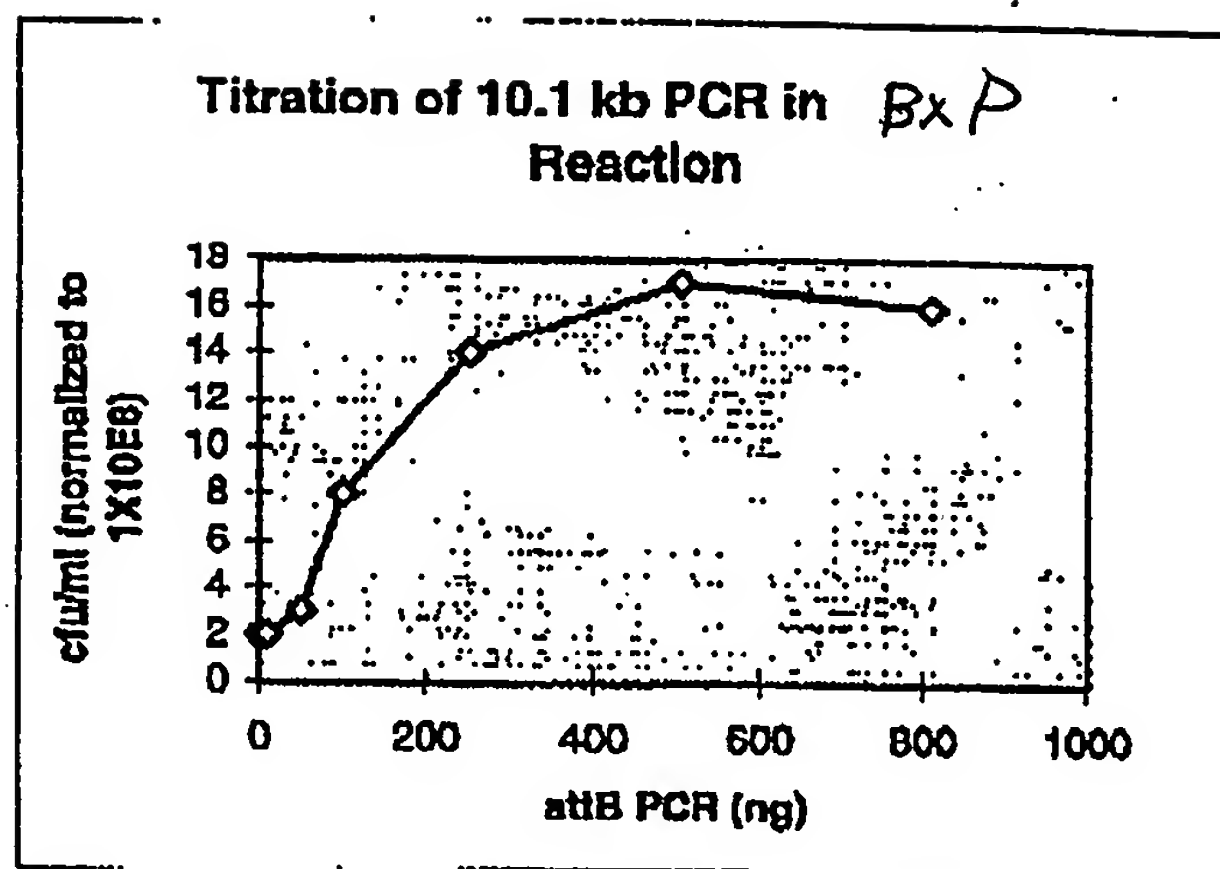


FIGURE 75-

# 10.1 kb PCR DNA Titration in Bx7 Reaction

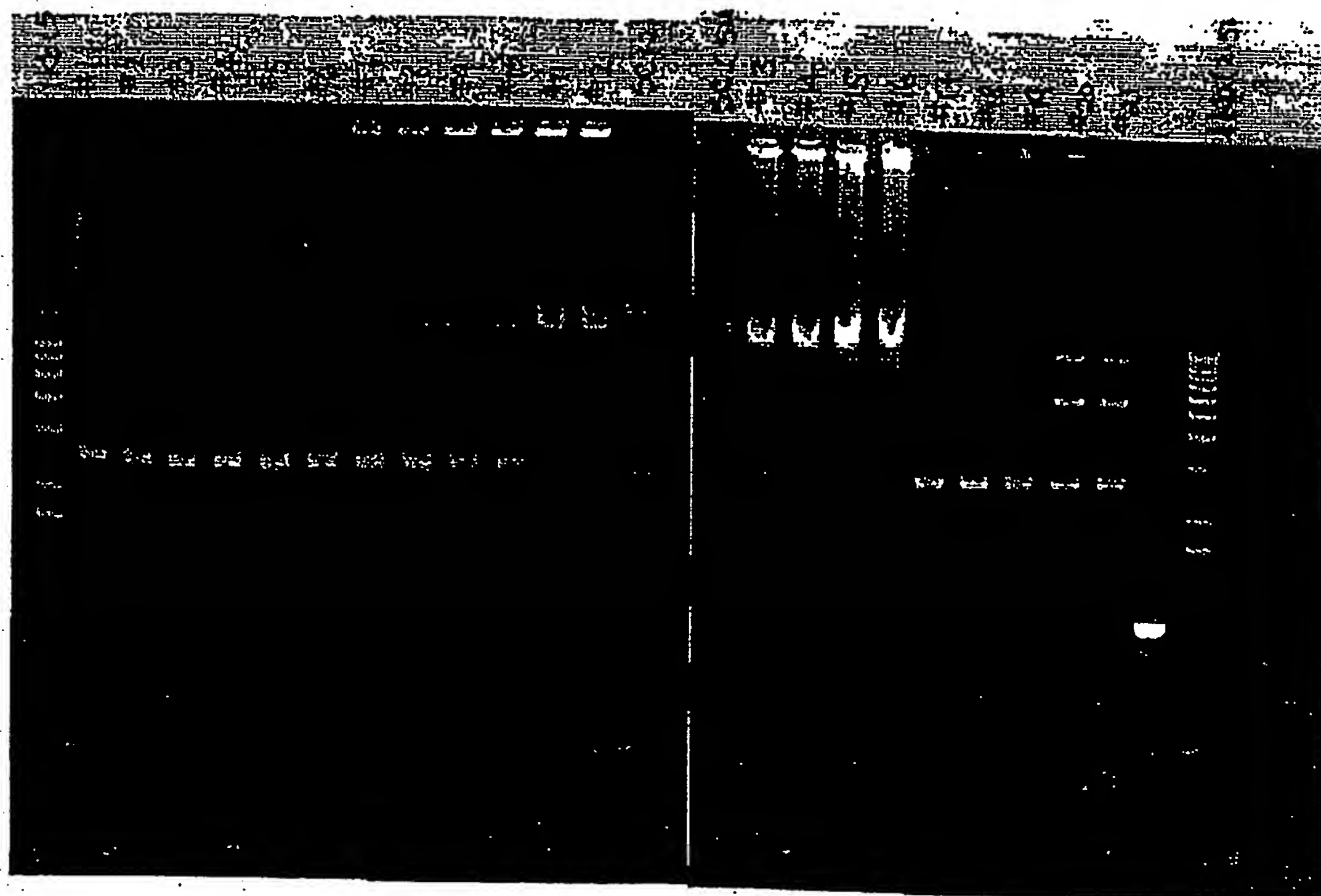


FIGURE 76

**Cloning of PCR Products of Different Sizes with the  
GATEWAY™ PCR Cloning System**

Size	fmols PCR DNA	ng PCR DNA	Cols/ml Transformation (pUC=10 <sup>8</sup> CFU/ml)	Correct Clones/Total Examined**
0.26 kb*	15	3	1223	10/10 (a)
	37.5	7.5	2815	
1.0 kb	15	10	507	49/50 (b)
	37.5	25	1447	
1.4 kb	15	14	271	48/50 (c)
	37.5	35	683	
3.4 kb	15	34	478	9/10 (a)
	37.5	85	976	
4.6 kb	15	46	190	10/10 (a)
	37.5	115	195	
6.9 kb	15	69	30 (235)**	47/50 (b)
	37.5	173	54 (463)**	

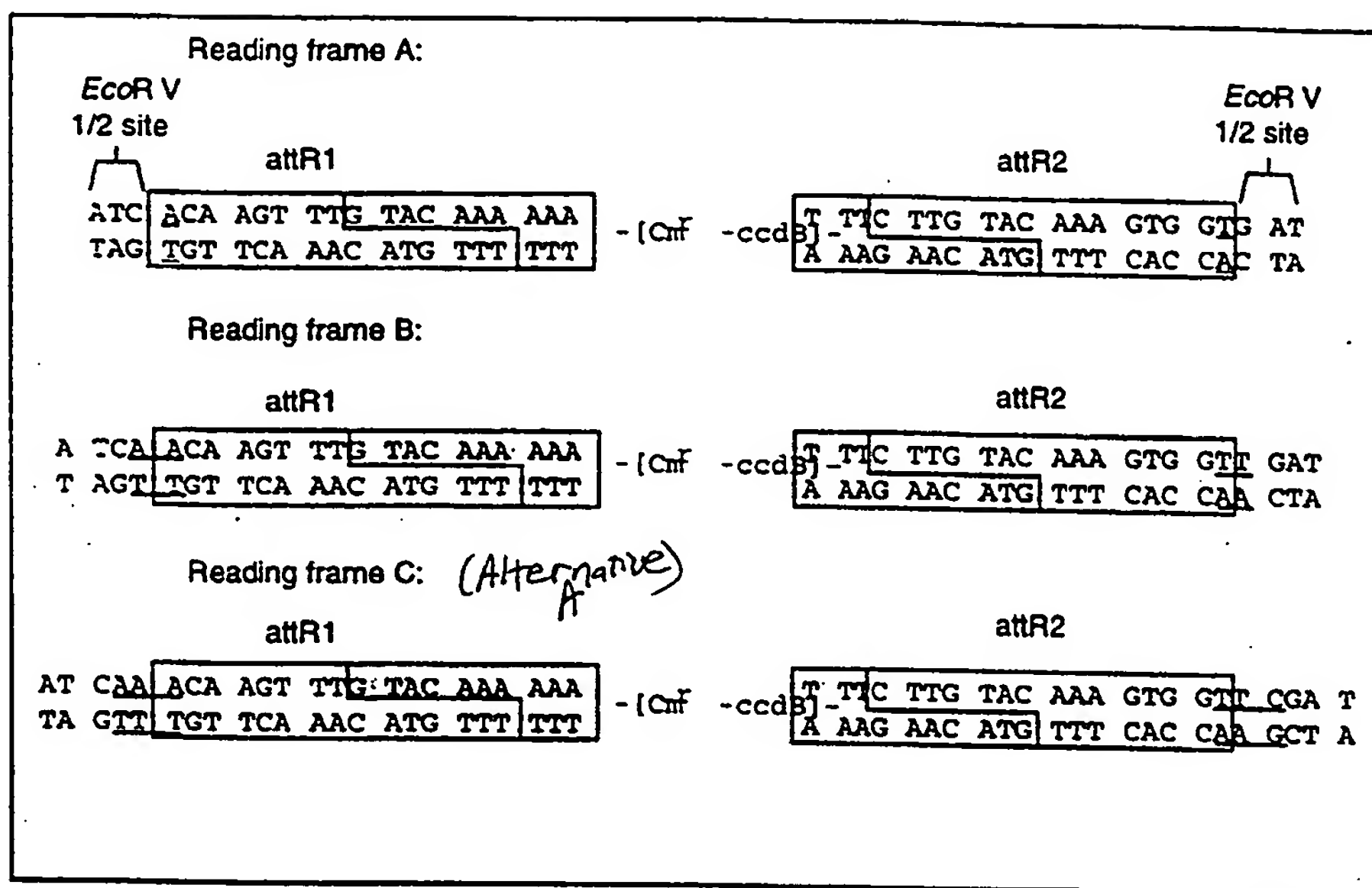
\*The 0.26 kb PCR product was used unpurified; all the others were purified by precipitation with PEG/MgCl<sub>2</sub> as described in the text of Example 9, to remove primer dimers potentially present. Standard incubations were for 60 min.

\*\*overnight incubation

- (a) DNA minipreps
- (b) ampR/kanR
- (c) tetR/kanR

**Figure 77**

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Reading frame C: (Alternative)  
B

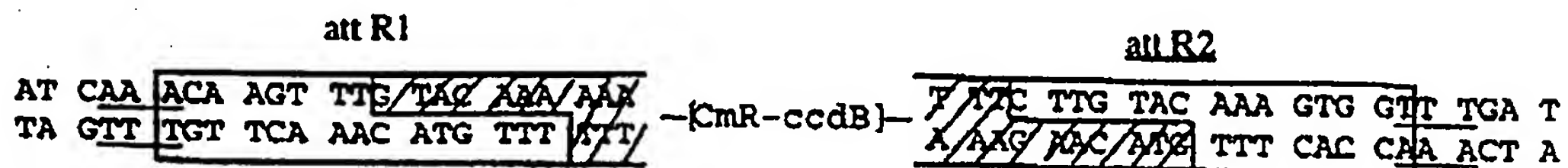


FIGURE 78



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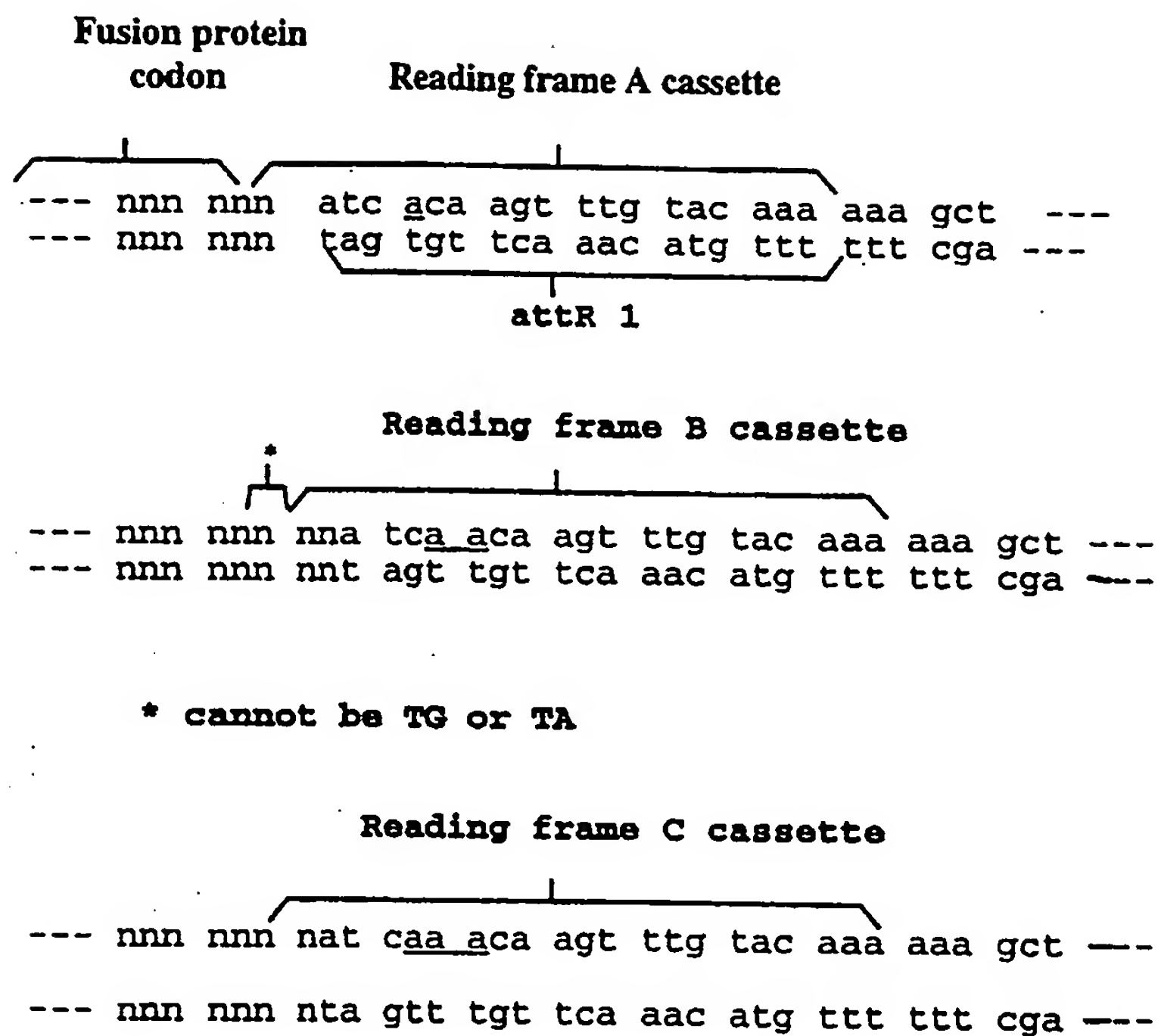


FIGURE 79

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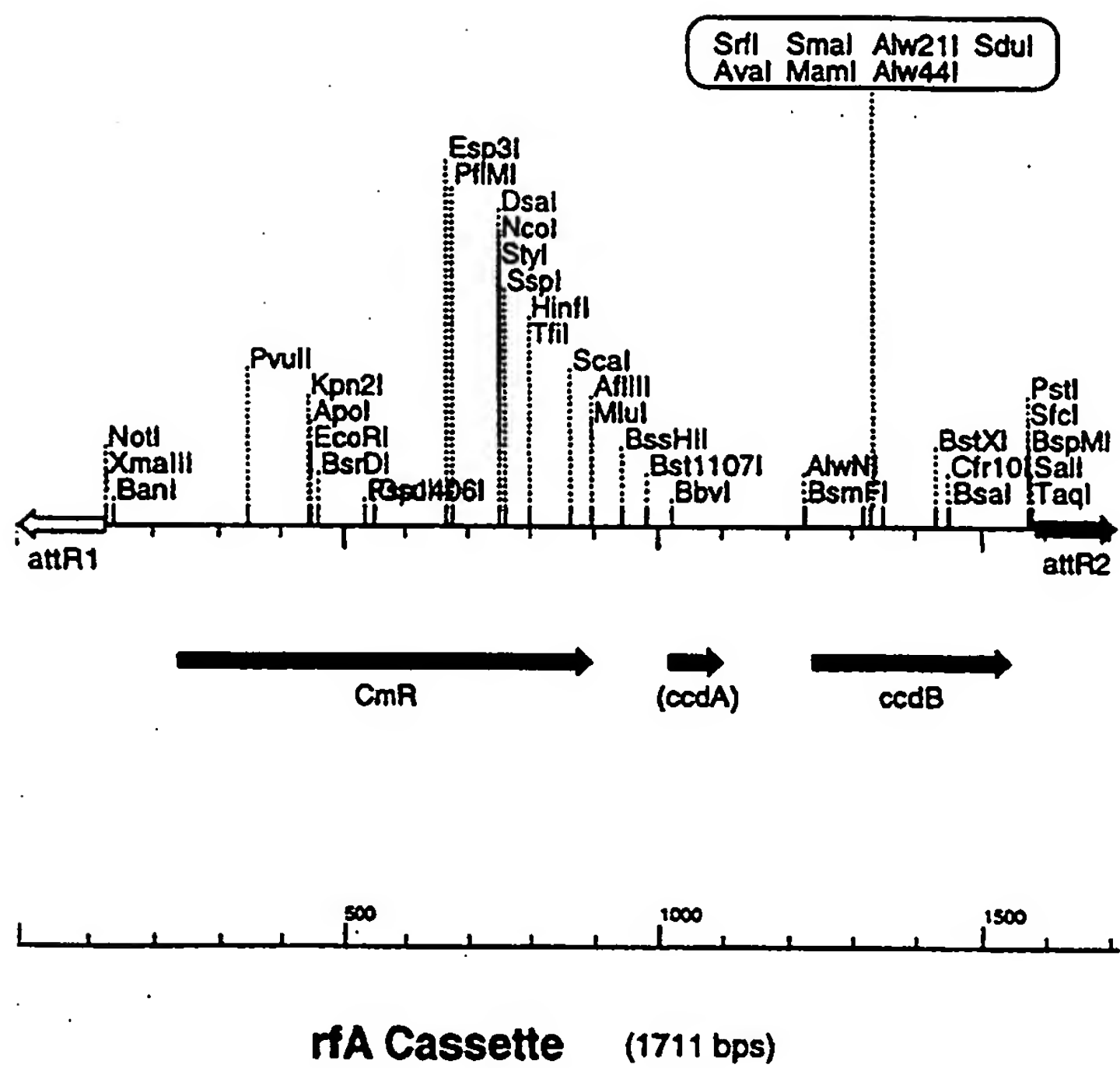


FIGURE 80

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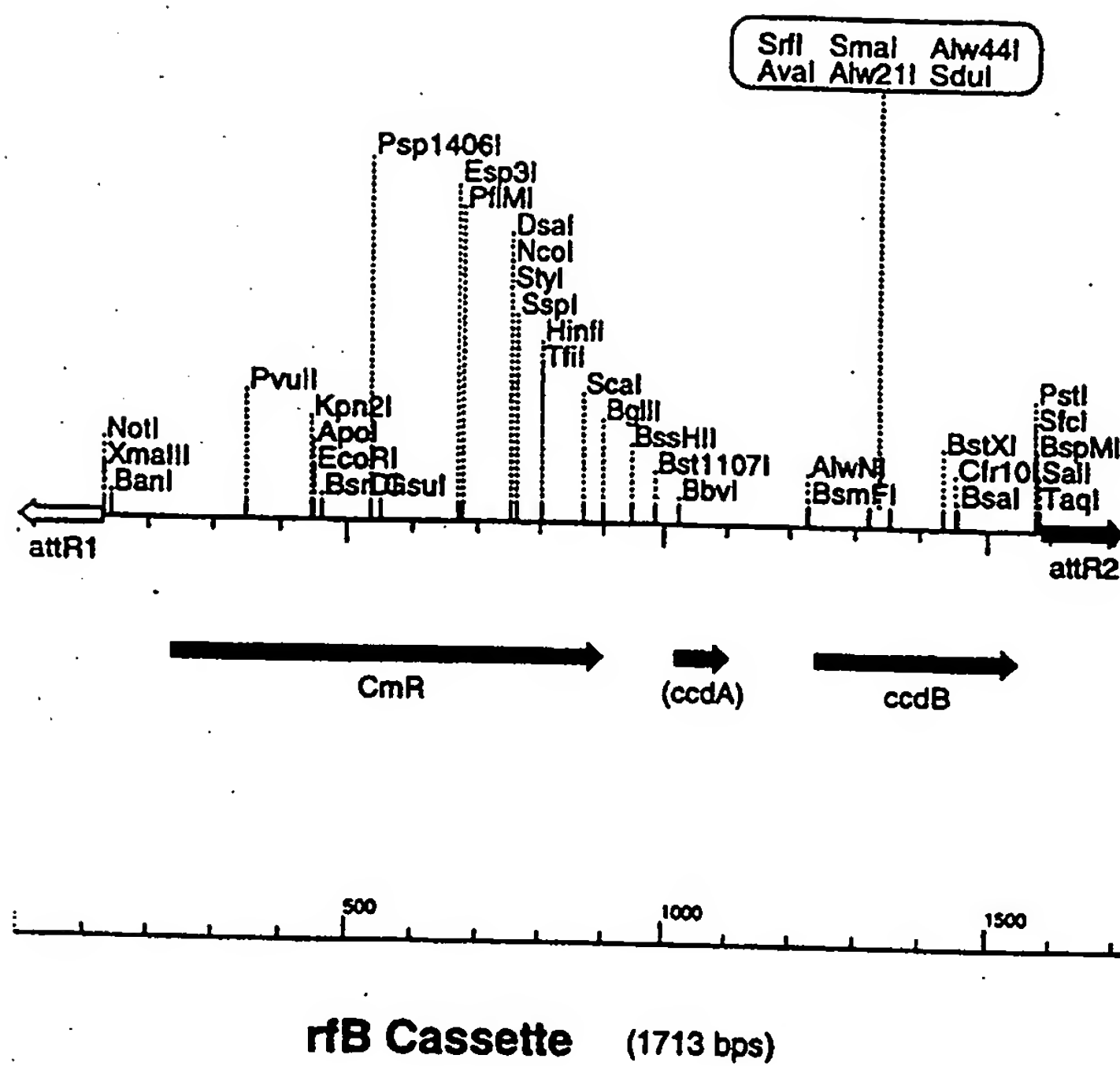
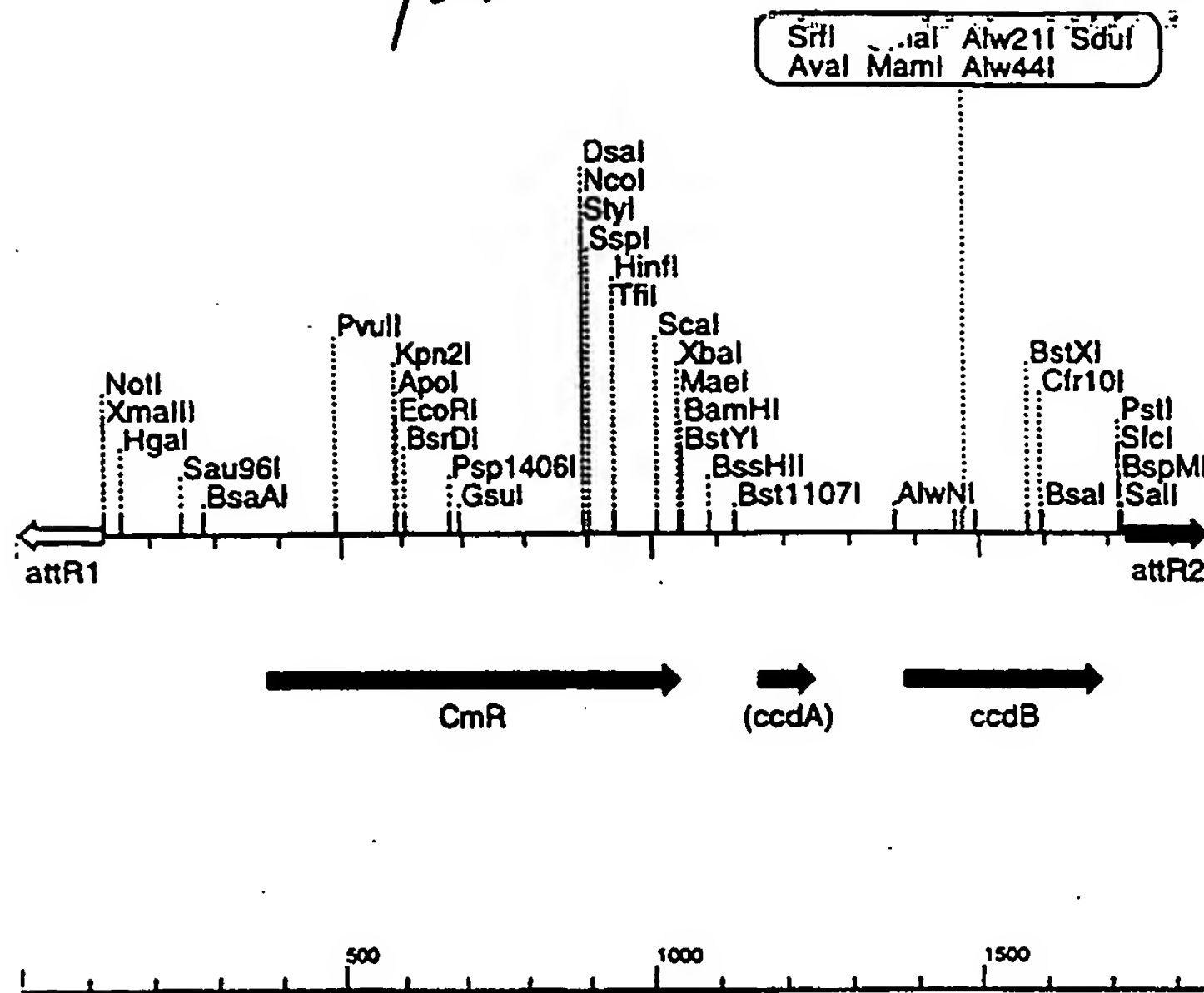


FIGURE 81

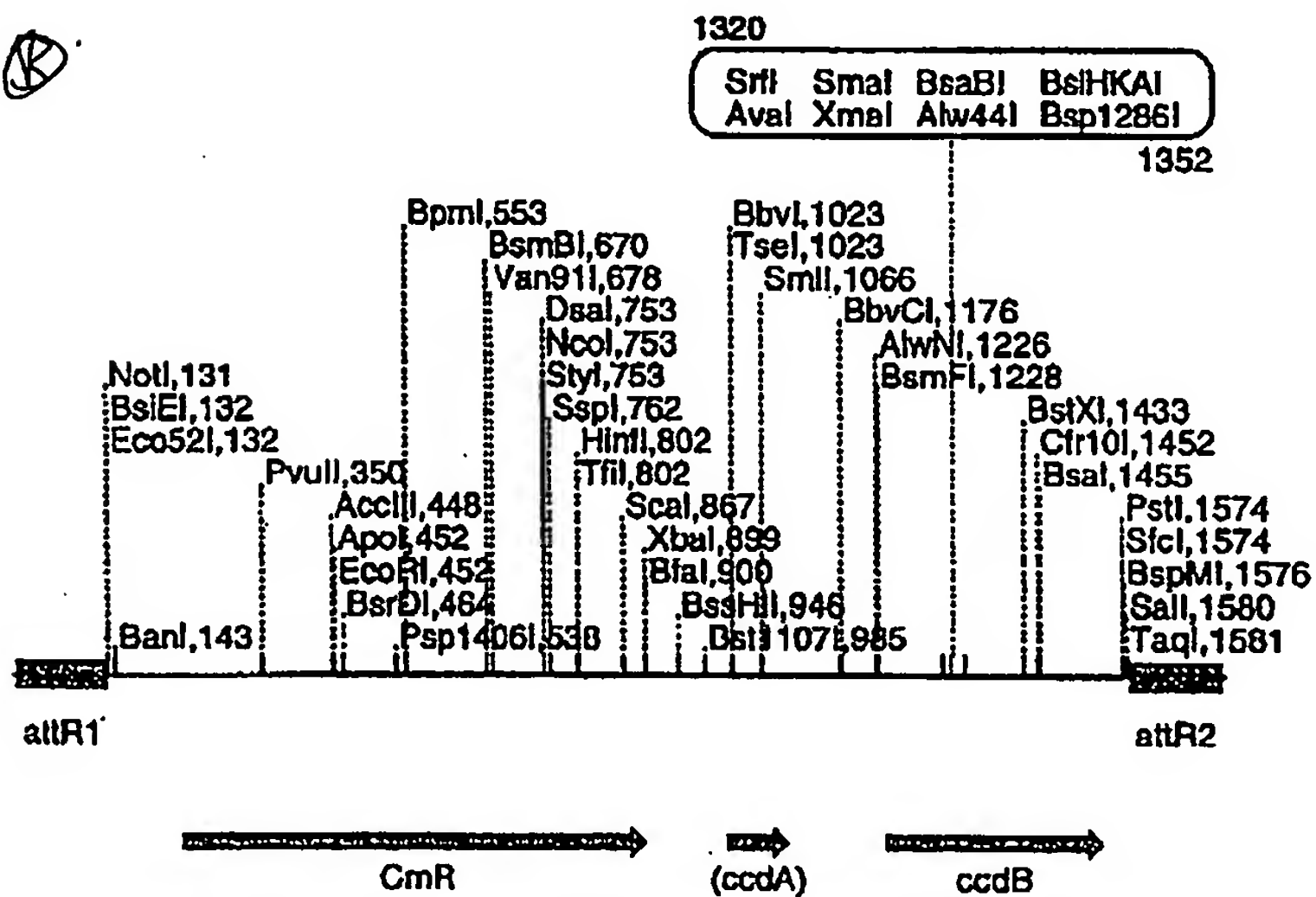
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(A)



rfc Cassette (1856 bps)

(B)



rfc cassette (1715 bps)

FIGURE 82

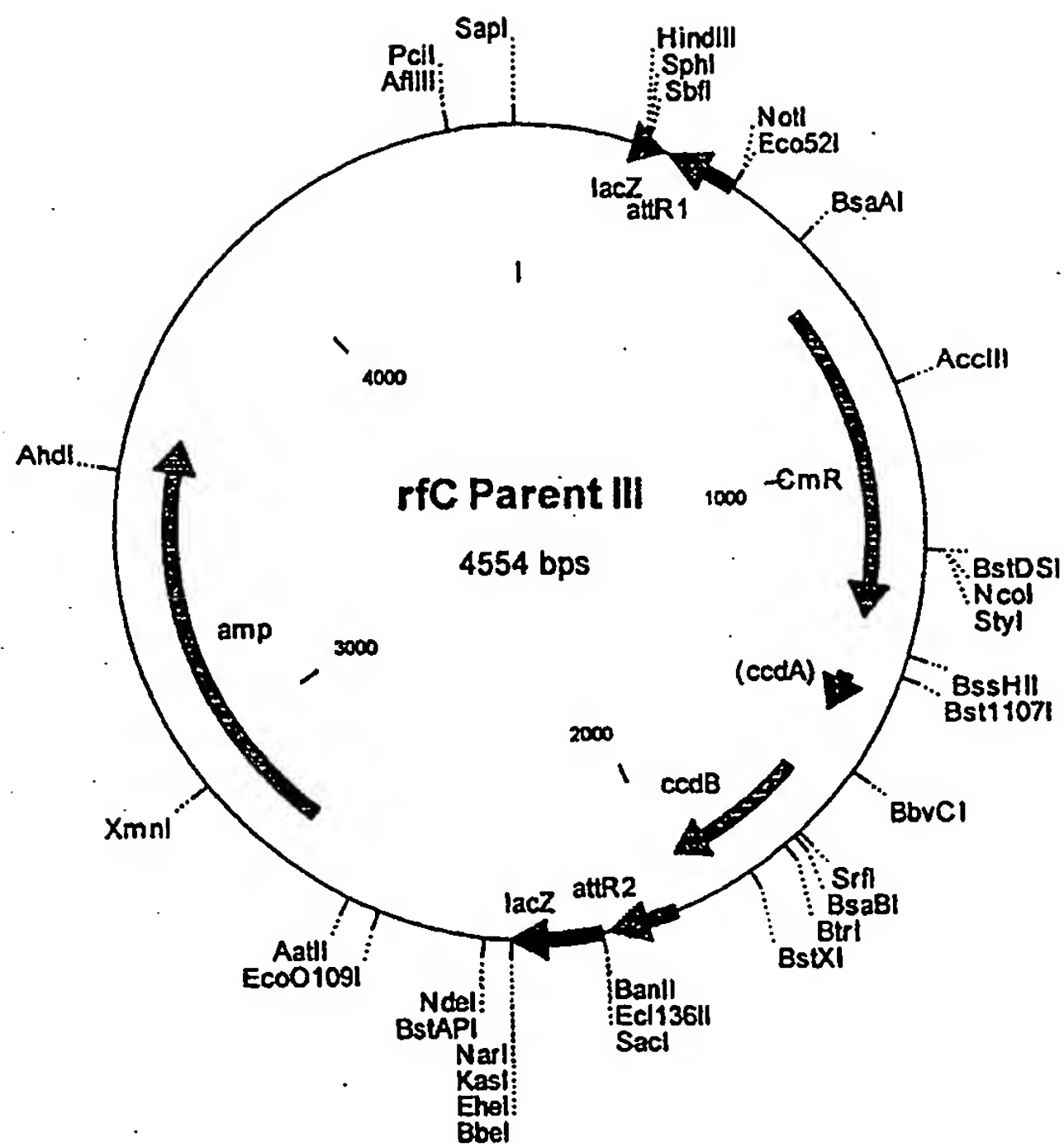


FIGURE 83A

## prfC Parent III 4554 bp

Location (Base Nos.)	Gene Encoded
410..286	attR1
660..1319	CmR
1439..1523	inactivated ccdA
1661..1966	ccdB
2007..2131	attR2
2753..3613	amp

```
1 GCGCCCAATA CGCAAACCGC CTCTCCCCGC GCGTTGGCCG ATTCATTAAT GCAGCTGGCA
61 CGACAGGTTT CCCGACTGGA AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT
121 CACTCATTAG GCACCCCAGG CTTTACACTT TATGCTTCCG GCTCGTATGT TGTGTGGAAT
181 TGTGAGCGGA TAACAATTTC ACACAGGAAA CAGCTATGAC CATGATTACG CCAAGCTTGC
241 ATGCCTGCAG GTCGACTCTA GAGGATCCCC GGGTACCGAT ATCAAACAAG TTTGTACAAA
301 AAAGCTGAAC GAGAAACGTA AAATGATATA AATATCAATA TATTAAATTA GATTTTGCAT
361 AAAAAACAGA CTACATAATA CTGTAAAACA CAACATATCC AGTCACTATG GCGGCCGCTA
421 AGTTGGCAGC ATCACCCGAC GCACTTTGCG CCGAATAAAT ACCTGTGACG GAAGATCACT
481 TCGCAGAATA AATAAATCCT GGTGTCCCTG TTGATACCGG GAAGCCCTGG GCCAACTTTT
541 GCGGAAAATG AGACGTTGAT CGGCACGTAA GAGGTTCCAA CTTTCACCAT AATGAAATAA
601 GATCACTACC GGGCGTATTT TTTGAGTTAT CGAGATTTTC AGGAGCTAAG GAAGCTAAAA
661 TGGAGAAAAA AATCACTGGA TATACCACCG TTGATATATC CCAATGGCAT CGTAAAGAAC
721 ATTTTGAGGC ATTTTCAGTCA GTTGCTCAAT GTACCTATAA CCAGACCGTT CAGCTGGATA
781 TTACGGCCTT TTTAAAGACC GTAAAGAAAA ATAAGCACAA GTTTTATCCG GCCTTTATTC
841 ACATTCTTGC CCGCCTGATG AATGCTCATC CGGAATTCCG TATGGCAATG AAAGACGGTG
901 AGCTGGTGAT ATGGGATAGT GTTCACCCTT GTTACACCGT TTTCCATGAG CAAACTGAAA
961 CGTTTTTCATC GCTCTGGAGT GAATACCACG ACGATTTCCG GCAGTTTCTA CACATATATT
1021 CGCAAGATGT GGCGTGTTAC GGTGAAAACC TGGCCTATTT CCCTAAAGGG TTTATTGAGA
1081 ATATGTTTTT CGTCTCAGCC AATCCCTGGG TGAGTTTCAC CAGTTTTGAT TTAAACGTGG
1141 CCAATATGGA CAACTTCTTC GCCCCGTTT TCACCATGGG CAAATATTAT ACGCAAGGCG
1201 ACAAGGTGCT GATGCCGCTG GCGATTGAGG TTCATCATGC CGTCTGTGAT GGCTTCCATG
1261 TCGGCAGAAT GCTTAATGAA TTACAACAGT ACTGCGATGA GTGGCAGGGC GGGGCGTAAT
1321 CTAGAGGATC CGGCTTACTA AAAGCCAGAT AACAGTATGC GTATTTGCGC GCTGATTTTT
1381 GCGGTATAAG AATATATACT GATATGTATA CCCGAAGTAT GTCAAAAAGA GGTGTGCTAT
1441 GAAGCAGCGT ATTACAGTGA CAGTTGACAG CGACAGCTAT CAGTTGCTCA AGGCATATAT
1501 GATGTCAATA TCTCCGGTCT GGTAAGCACA ACCATGCAGA ATGAAGCCCC TCGTCTGCGT
1561 GCCGAACGCT GGAAAGCGGA AAATCAGGAA GGGATGGCTG AGGTGCCCCG GTTTATTGAA
1621 ATGAACGGCT CTTTGTCTGA CGAGAACAGG GACTGGTGAA ATGCAGTTTA AGGTTTACAC
1681 CTATAAAGA GAGAGCCGTT ATCGTCTGTT TGTGGATGTA CAGAGTGATA TTATTGACAC
1741 GCGCGGGCGA CGGATGGTGA TCCCCCTGGC CAGTGACCGT CTGCTGTCAG ATAAAGTCTC
1801 CCGTGAACCT TACCCGGTGG TGCATATCGG GGATGAAAGC TGGCGCATGA TGACCACCGA
1861 TATGGCCAGT GTGCCGGTCT CCGTTATCGG GGAAGAAGTG GCTGATCTCA GCCACCGCGA
1921 AAATGACATC AAAAACGCCA TTAACCTGAT GTTCTGGGGA ATATAAATGT CAGGCTCCGT
1981 TATACACAGC CAGTCTGCAG GTCGACCATA GTGACTGGAT ATGTTGTGTT TTACAGTATT
2041 ATGTAGTCTG TTTTATATGC AAAATCTAAT TTAATATATT GATATTTATA TCATTTTACG
2101 TTTCTCGTTC AGCTTCTTGG TACAAAGTGG TTCGATATCG GTACCGAGCT CGAATTCATC
2161 GGCCGTCGTT TTACAACGTC GTGACTGGGA AAACCCTGGC GTTACCCAAC TTAATCGCCT
2221 TGCAGCACAT CCCCCTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC
2281 TTCCCAACAG TTGCGCAGCC TGAATGGCGA ATGGCGCCTG ATGCGGTATT TTCTCCTTAC
2341 GCATCTGTGC GGTATTTTAC ACCGCATATG GTGCACTCTC AGTACAATCT GCTCTGATGC
2401 CGCATAGTTA AGCCAGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG
2461 TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC TCCGGGAGCT GCATGTGTCA
2521 GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGACGAAAG GGCCTCGTGA TACGCCTATT
2581 TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG
2641 AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT
2701 CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTATGAGTAT
2761 TCAACATTTT CGTGTCGCCC TTATTCCTT TTTTGGCGCA TTTTGCCTTC CTGTTTTTGC -
```



2821 TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG  
2881 TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG  
2941 TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTATTGA  
3001 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT AACTATTCT CAGAATGACT TGGTTGAGTA  
3061 CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC  
3121 TGCCATAACC ATGAGTGATA AACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC  
3181 GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACGCGC TTGATCGTTG  
3241 GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT GACACCACGA TGCCTGTAGC  
3301 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG CTTCCCGGCA  
3361 ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT  
3421 TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT  
3481 CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT ACACGACGGG  
3541 GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT  
3601 TAAGCATTGG TAAGTGTGAG ACCAAGTTTA CTCATATATA CTTTAGATTG ATTTAAAACT  
3661 TCATTTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAT  
3721 CCCTTAACGT GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC  
3781 TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA AACCACCGCT  
3841 ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAAC TGG  
3901 CTTCAAGACA GCGCAGATAC CAAATACTGT CCTTCTAGTG TAGCCGTAGT TAGGCCACCA  
3961 CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC  
4021 TGCTGCCAGT GGCATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA  
4081 TAAGGCGCAG CGGTGCGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT TGGAGCGAAC  
4141 GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCTATGA GAAAGCGCCA CGCTTCCCGA  
4201 AGGGAGAAAG GCGGACAGGT ATCCGGTAAG CGGCAGGGTC GGAACAGGAG AGCGCACGAG  
4261 GGAGCTTCCA GGGGGAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG  
4321 ACTTGAGCGT CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG  
4381 CAACGCGGCC TTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCCTTCC  
4441 TCGGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC  
4501 TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG AAGA

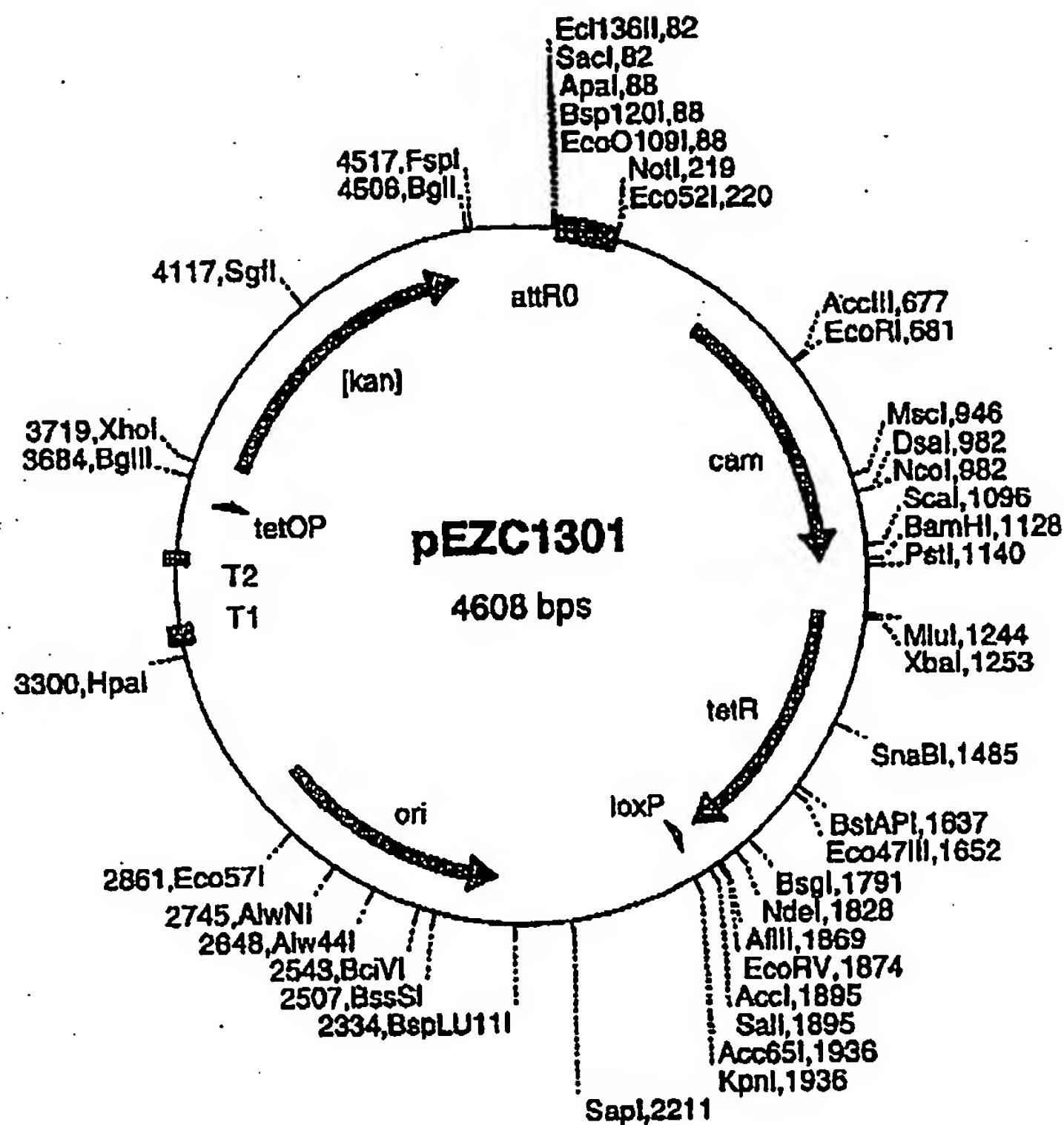


FIGURE 84

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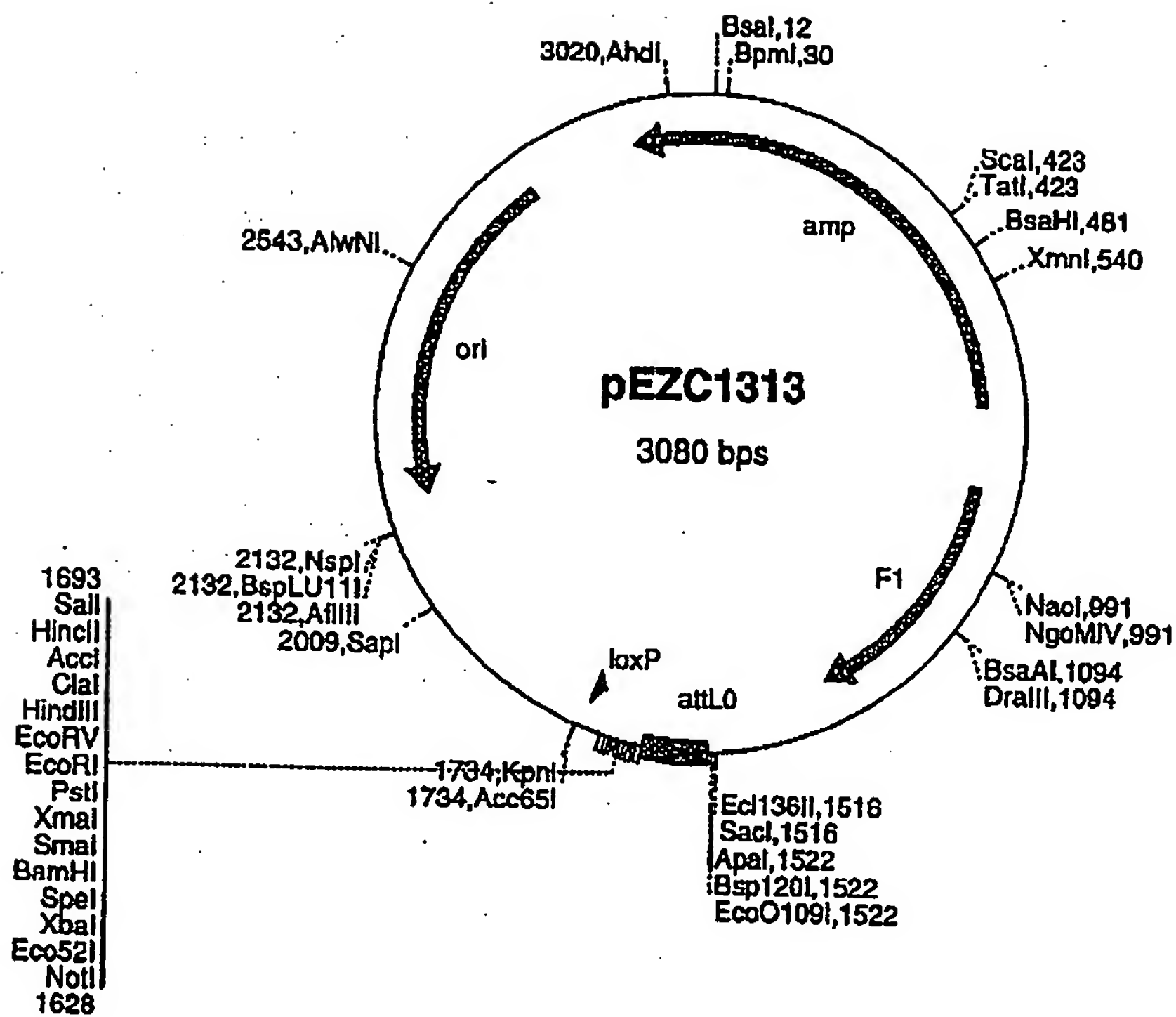


FIGURE 85

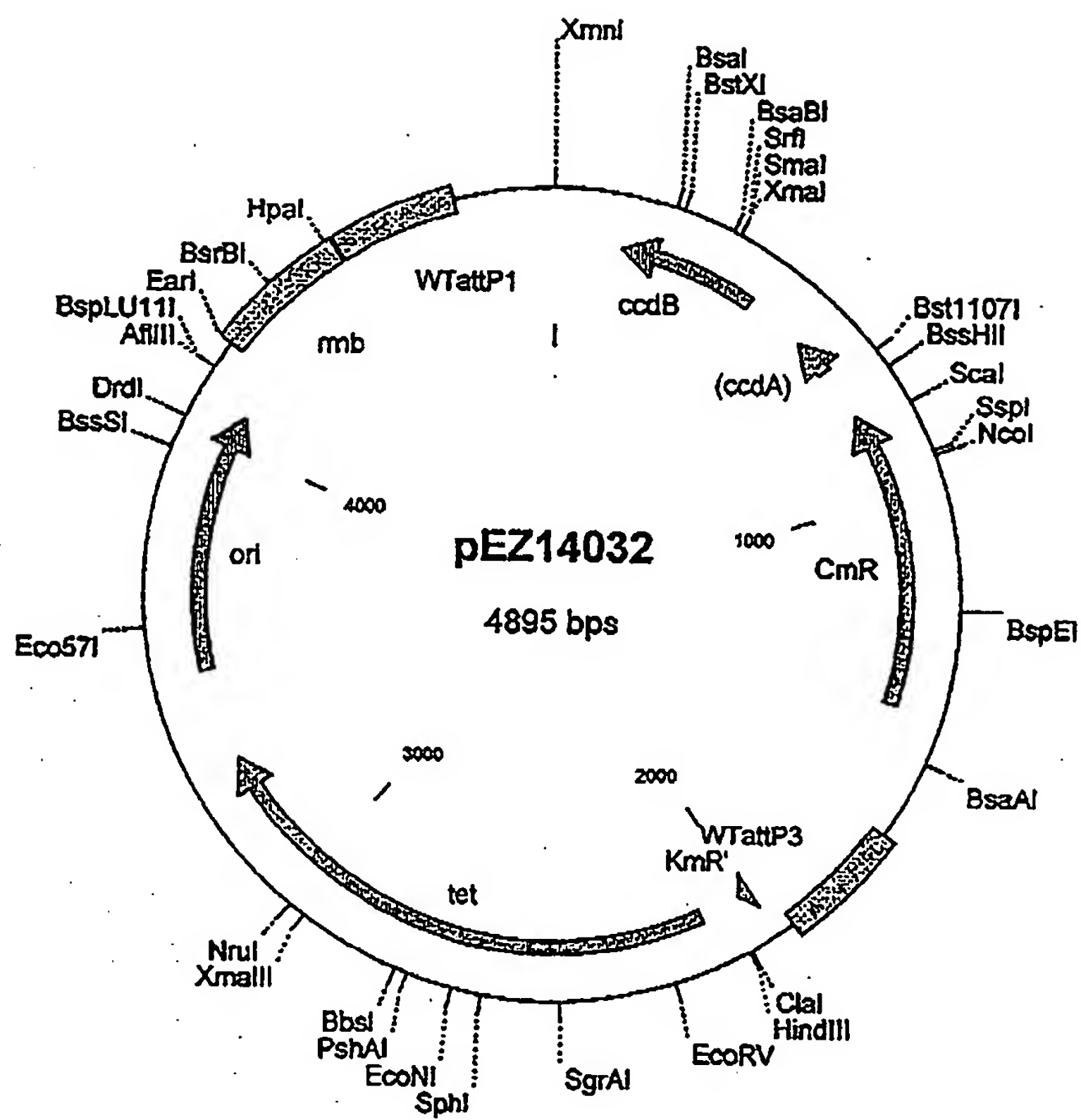


FIGURE 86

FIGURE 87

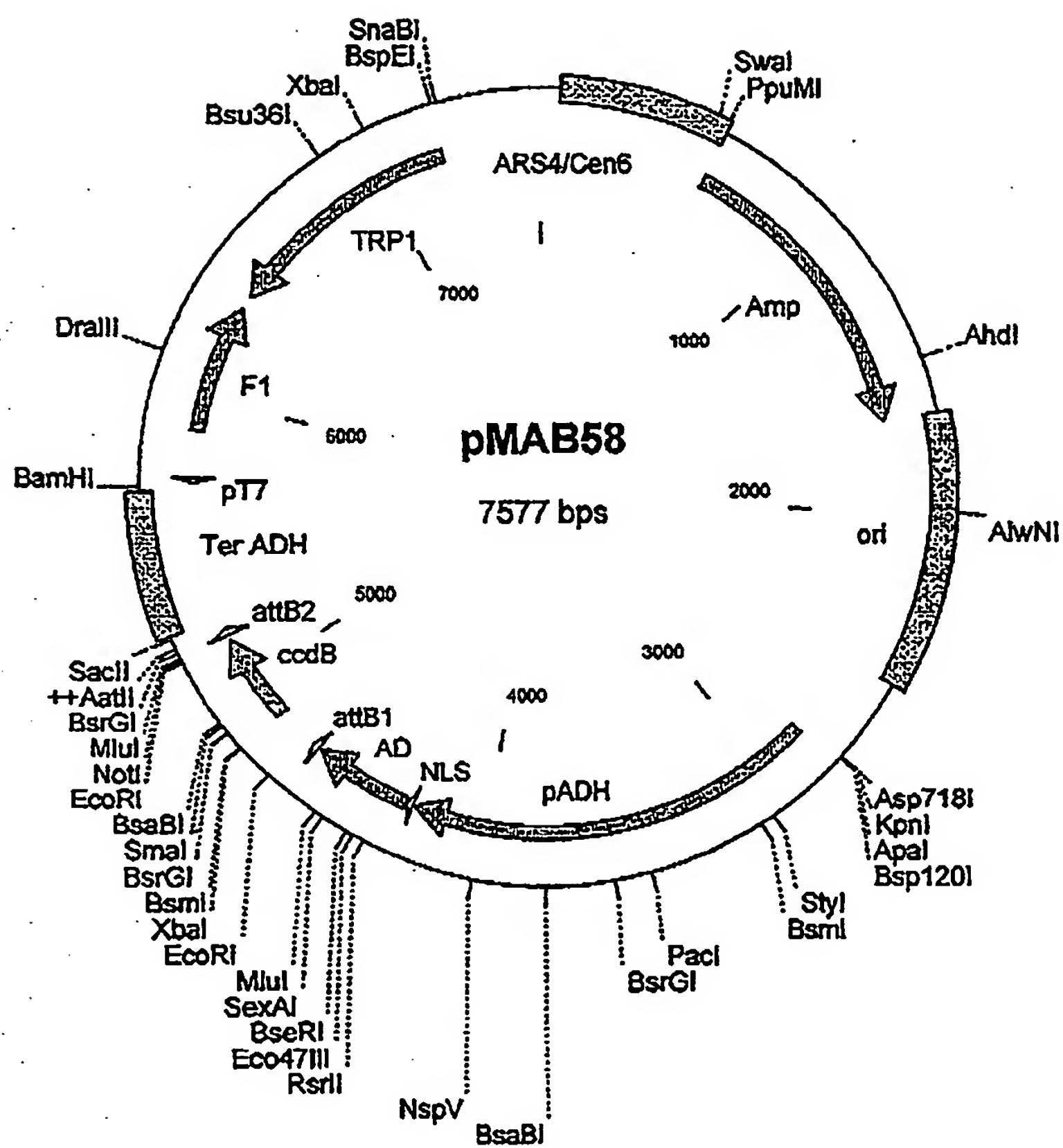
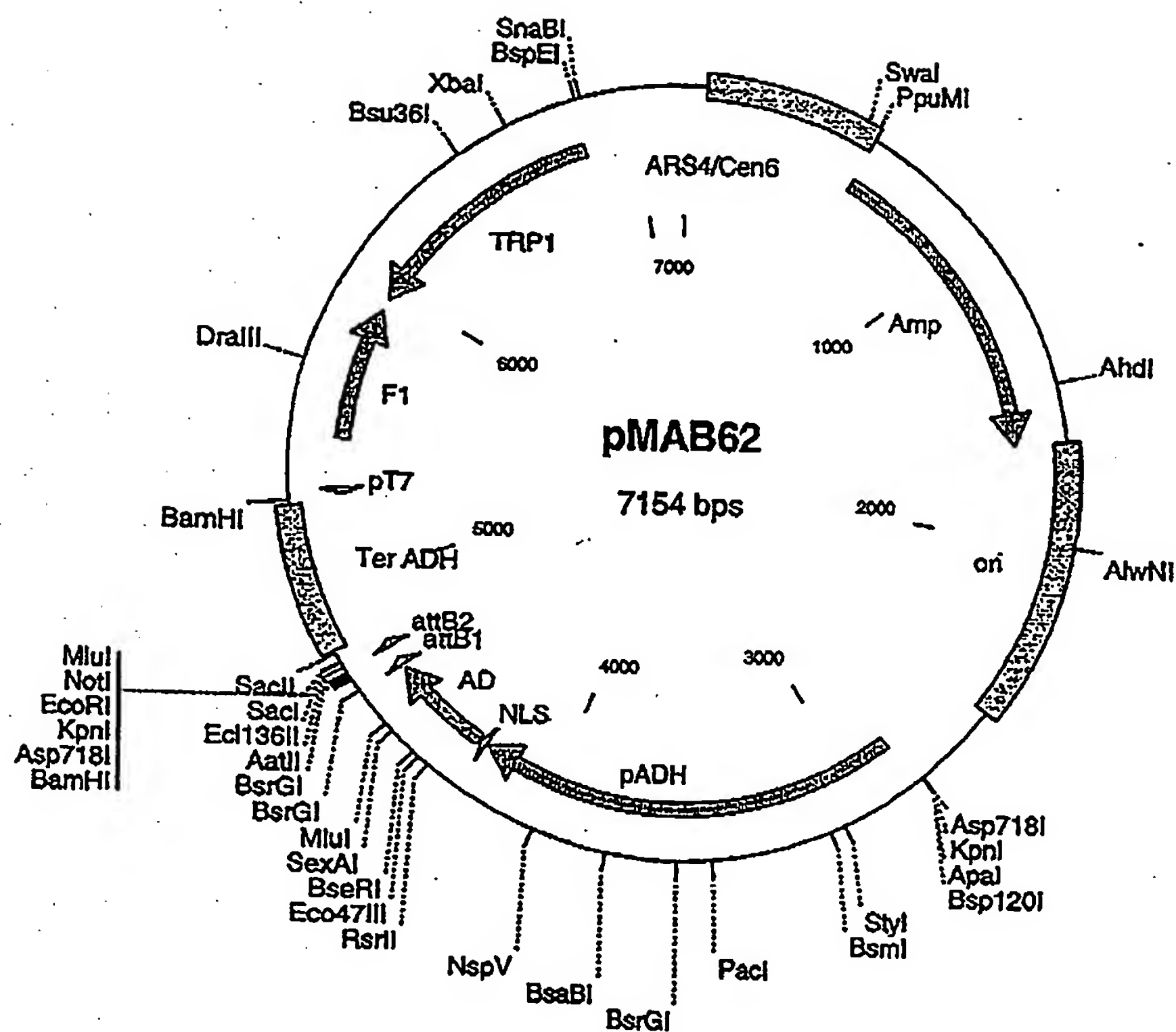
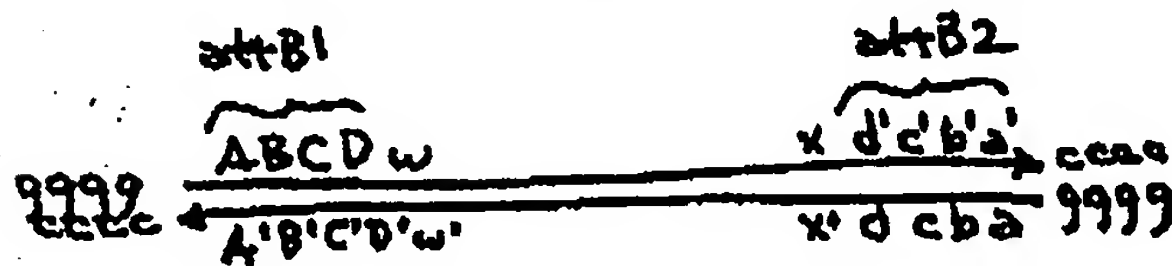
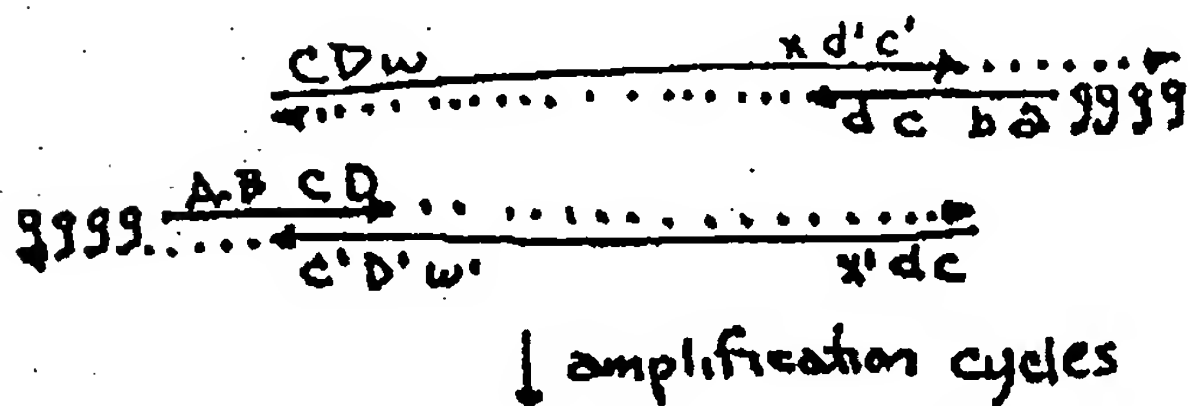
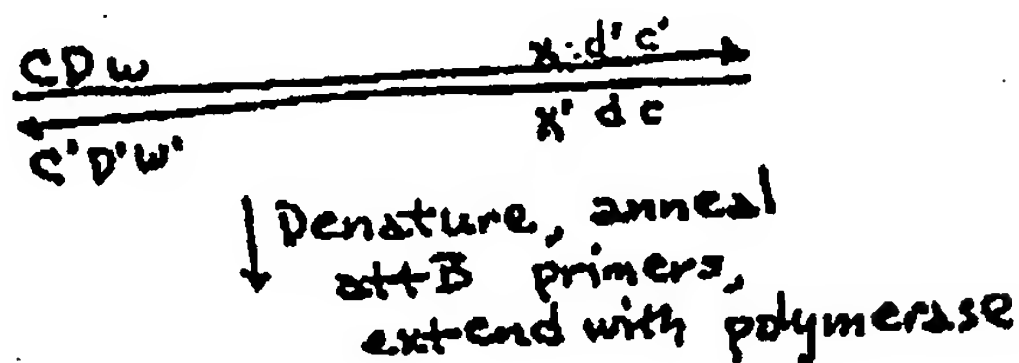
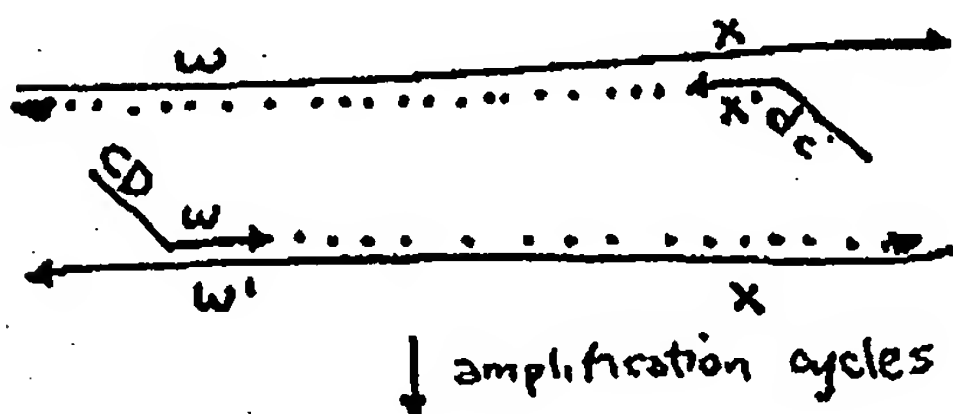
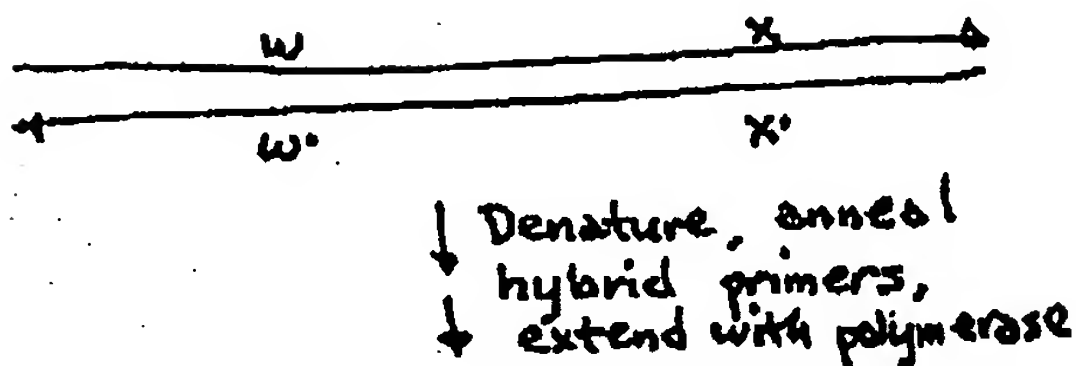


FIGURE 88





DNA to be amplified (5' → 3'):



attB1 primer:  
9999 ABCD →

attB2 primer:  
9999 abca →

Hybrid primers (part attB, part gene specific):

CDw →  
cd x' →

FIGURE 89

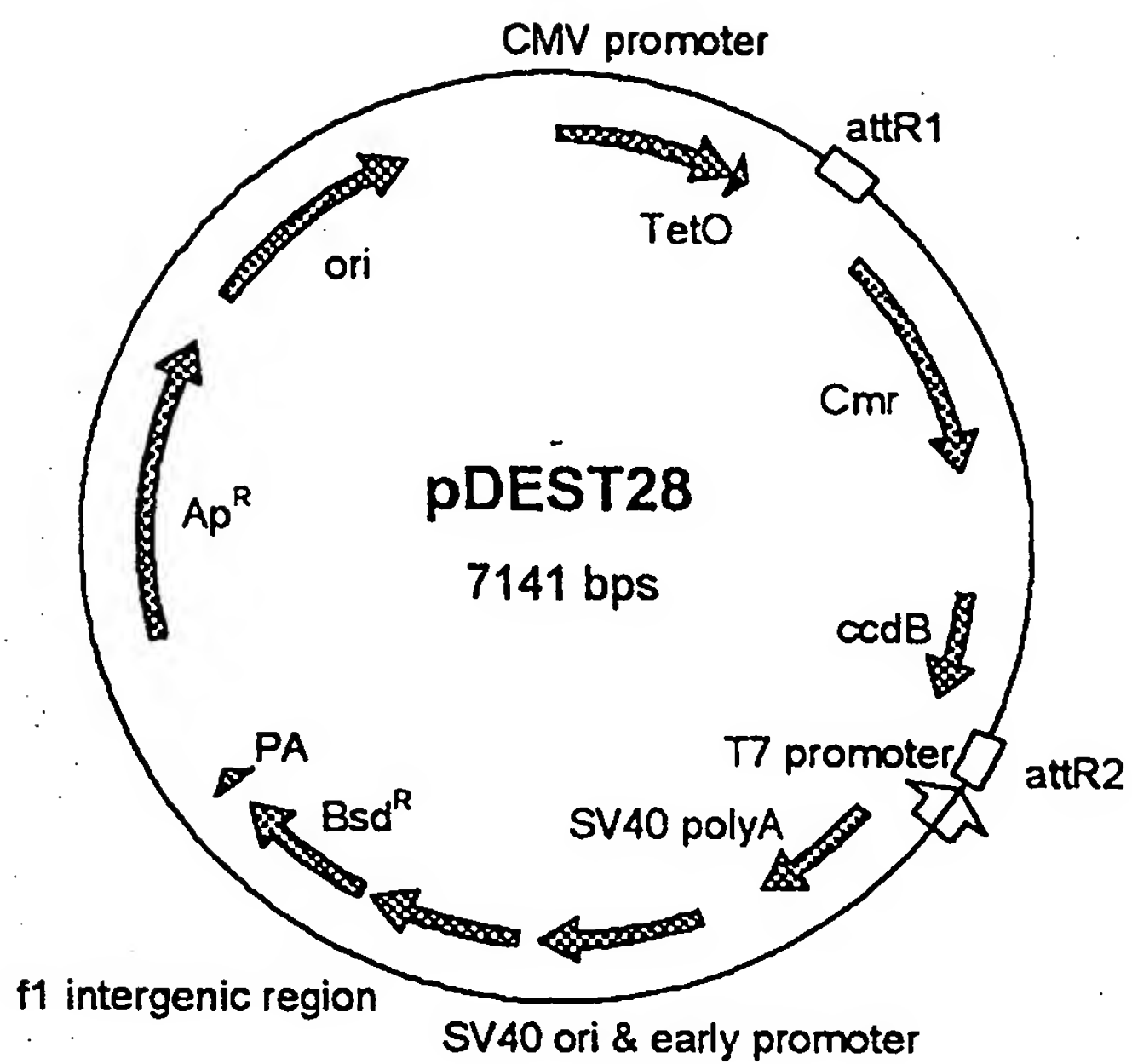


FIGURE 90A

pDEST28 7141 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC  
CGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGATTTCGAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT  
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAAGCTG  
AACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGGCGGCCGCATTAGGCAC  
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGAGTTAGGATCC  
GGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCAC  
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCA  
ATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAA  
AAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGATGAATGCTCA  
TCCGGAATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCAACC  
TTGTTACACCGTTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCA  
CGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAA  
CCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCTGCTCAGCCAATCCCTG  
GGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGT  
TTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA  
GGTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGAATTACAACA  
GTACTGCGATGAGTGGCAGGGCGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG  
ATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTA  
TACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC  
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA  
CAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGG  
AAGGGATGGCTGAGGTGCGCCGGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACA  
GGGACTGGTGAAATGCAGTTTAAGGTTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTG  
TTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTG  
GCCAGTGACGCTGCTGTGCTGATATAAGTCTCCCGTGAACTTTACCCGGTGGTGATATC  
GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC  
GGGGAAGAAGTGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACTTG  
ATGTTCTGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCA  
TAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAAGTCTGTTTTTTATGCAAAATCTA  
ATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTTCAGCTTTCTTGTACAAAGT  
GGTTGATGGGCGGCGCTCTAGAGGGCCCAAGCTTACGCGTGCATGCGACGTCATAGCTC  
TCTCCCTATAGTGAGTCTGATTATAAGCTAGGCACTGGCCGTCTGTTTTACAACGTCGTGA  
CTGGGAAACTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA  
ATTGGACAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGT  
ATAATGTGTTAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTTGCTTACTGAGTATGA  
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTATTTTAGATTCA  
CAGTCCCAAGGCTCATTTTCAGGCCCTCAGTCCTCACAGTCTGTTTCATGATCATAATCAG  
CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAA  
CCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGG  
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCTACTGCATTC  
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AGGCCCCGACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGC  
CCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACAC  
TTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTCTCGCCACGTTTCG  
CCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT-

Figure 90B

TACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGC  
CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT  
TGTTCCAAACTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATTTATAAGGGA  
TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTAAACAAATATTTAACGCGA  
ATTTTAACAAATATTAACGTTTACAATTTTCGCTGATGCGGTATTTTCTCCTTACGCAT  
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT  
CTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGT  
GTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC  
ATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA  
TGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCCATCC  
CGCCCCCTAACTCCGCCCCAGTTCCGCCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA  
TTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCT  
TTTTTGGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACACAACAGTCTCGAACT  
TAAGACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGC  
TACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAG  
CGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGA  
ACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGC  
GATCGGAAATGAGAACAGGGGCATCTTGAGCCCCCTGCGGACGGTGCCGACAGGTGCTTCT  
CGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGT  
TGGGATTCTGTAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGCACTTCGTGGCCG  
AGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATA  
TCTTTATTTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATC  
CGCGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGA  
CACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC  
AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTTACCCTCATCACCG  
AAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTGATGATA  
ATAATGGTTTTCTTAGACGTGAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT  
TGTTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAA  
ATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCCCTT  
ATTCCTTTTTTTGCGGCATTTTGCCTTCTGTTTTTTGCTCACCCAGAAACGCTGGTGAAA  
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAAC  
AGCGGTAAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCAATGATGAGCACTTTT  
AAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGT  
CGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCAT  
CTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAC  
ACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTG  
CACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC  
ATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAA  
CTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG  
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCT  
GATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGAT  
GGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAA  
CGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAAGCATTGGTAACTGTCAGAC  
CAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATC  
TAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTT  
CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTG  
CGCGTAATCTGCTGCTTGCAAAACAAAAAACACCGCTACCAGCGGTGGTTTGTTTGCCG  
GATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCA  
AATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCG  
CCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCG  
TGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGGCTGA  
ACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATAC  
CTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTAT  
CCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCC  
TGGTATCTTTATAGTCCTGTGCGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA  
TGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCTTTTTTACGGTTC  
CTGGCCTTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTG  
GATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACCCGAACGACCGAG-

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CGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCC  
GCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGA  
AGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAAT  
AAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACC  
ATTATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTA  
G

FIGURE 90D

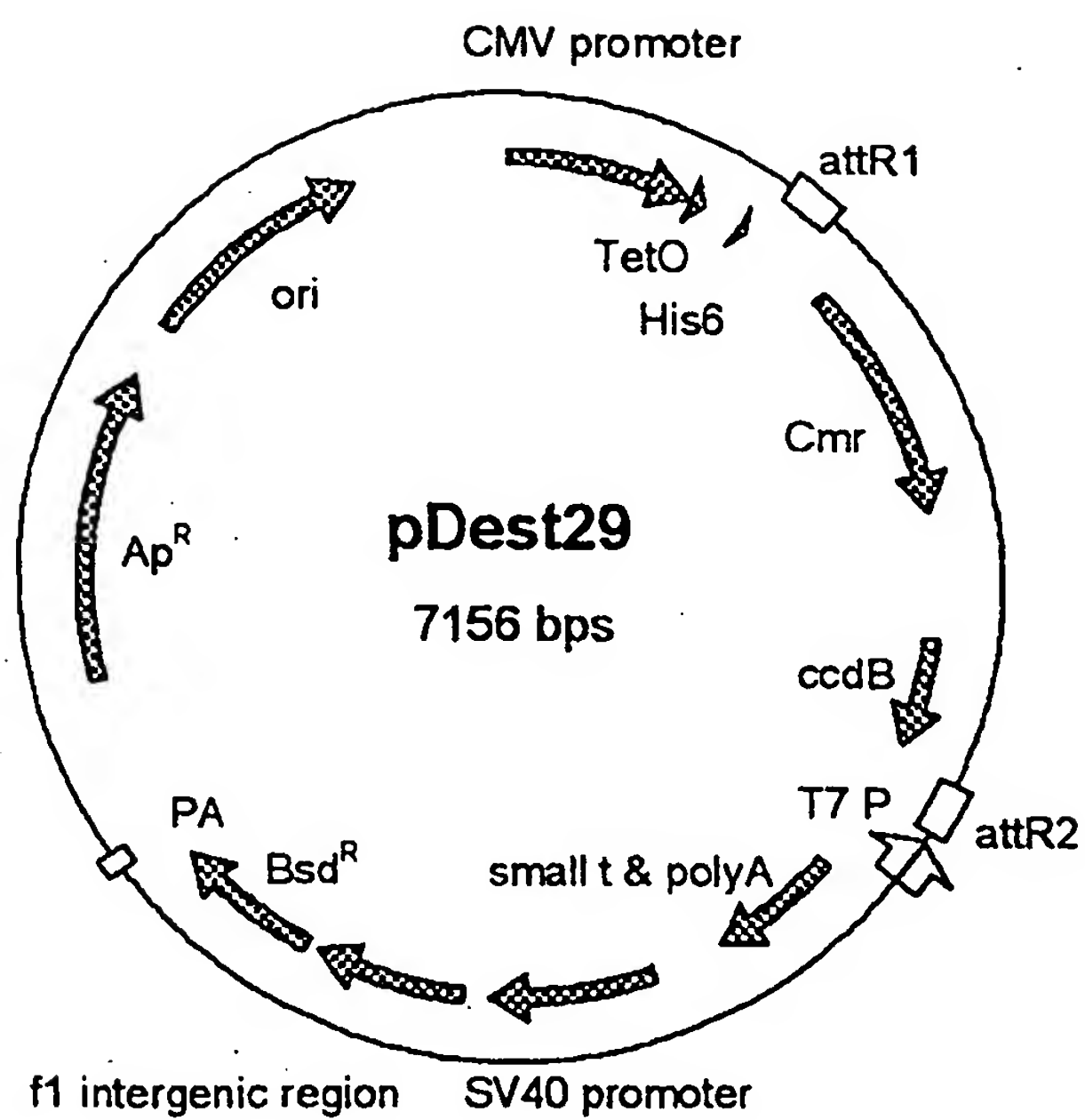


FIGURE 91 A



pDEST29 7156 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCC  
CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCTGTAACAACTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC  
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAGT  
TTGTACAAAAAAGCTGAACGAGAAACGTAAATGATATAAATATCAATATATTAAATTAG  
ATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACATATCCAGTCACTATGG  
CGGCCGCATTAGGCACCCCAAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA  
TTTTGAGTTAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCTAAATGGAGAAAAAA  
TCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT  
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTACGCTGGATATTACGGCCTTTT  
TAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTACATTCTTGCCC  
GCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATAT  
GGGATAGTGTTACCCCTTGTTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGC  
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGG  
CGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCG  
TCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACA  
ACTTCTTCGCCCCCGTTTTTACCATGGGCAATATTATACGCAAGGCGACAAGGTGCTGA  
TGCCGCTGGCGATTACAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGC  
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAACGCGTGGATCCG  
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA  
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT  
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC  
TCCGGTCTGGTAAGCACAACCATGCAGAAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGG  
AAAGCGGAAAATCAGGAAGGGATGGCTGAGGTCCGCCGGTTTATTGAAATGAACGGCTCT  
TTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGGTTTACACCTATAAAAGAGA  
GAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCGACG  
GATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTGATGATAAAGTCTCCCGTGAACTTTA  
CCCGGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT  
GCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAA  
AAACGCCATTAAACCTGATGTTCTGGGGAATATAAATGTCAGGCTCCGTTATACACAGCCA  
GTCTGCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT  
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTTACG  
CTTTCTTGTAACAAGTGGTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGAT  
GCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGT  
TTTACAACGTCGTGACTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT  
CTGTGGTGATGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATAT  
AAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT  
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG  
TGTAATTTTAGATTACAGTCCCAAGGCTCATTTACAGGCCCTCAGTCCTCACAGTCTGTT  
CATGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCC  
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT  
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAATTTTACAAATAAAGCATT  
TTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTG  
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCT  
GGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG  
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCT  
TTCTCGCCACGTTTCGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

Figure 91B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCAC  
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT  
TTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT  
TTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC  
AAATATTTAACGCGAATTTTAACAAAATATTAACGTTTACAATTTTCGCCTGATGCGGTAT  
TTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCAT  
GGCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCGGAAAGAACC  
AGCTGTGGAATGTGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCC  
CAGCAGGCAGAAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC  
TAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCCATTCTCCGCCCCATGGCT  
GACTAATTTTTTTTATTTATGTCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGA  
AGTAGTGAGGAGGCTTTTTTGGAGGCCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA  
CAACAGTCTCGAACTTAAGACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCAT  
TGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAG  
CGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGG  
GGGACCTTGTGCAGAACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCT  
GACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCTGCGGACGGTG  
CCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGG  
ACAGCCGACGGCAGTTGGGATTCTGTAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTA  
AGCACTTCGTGGCCGAGTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAT  
GGCCGCAATAAAATATCTTTATTTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCG  
ATAGCGATAAGGATCCGCGTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAG  
TTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC  
CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTT  
TCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAG  
GTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTG  
CGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGA  
CAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACAT  
TTCCGTGTGCCCCTTATTCCTTTTTTTCGGGCATTTTGCCTTCTGTTTTTGTCTCACCCA  
GAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATC  
GAACTGGATCTCAACAGCGGTAAAGATCCTTGAGAGTTTTTCGCCCCGAAGAAGCTTTTCCA  
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG  
CAAGAGCAACTCGGTGCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCA  
GTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATA  
ACCATGAGTGATAAACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAG  
CTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCG  
GAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAGATGCCTGTAGCAATGGCA  
ACAACGTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTA  
ATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCCTTCTGCGCTCGGCCCTTCCGGCT  
GGCTGGTTTATTTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA  
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAG  
GCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCAT  
TGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTT  
TAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAATCCCTTAA  
CGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGA  
GATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAACAAAAAACCACCGCTACCAGCG  
GTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGC  
AGAGCGCAGATACCAAATACTGTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAG  
AACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCC  
AGTGGCGATAAGTCGTGTCTTACCGGGTGGACTCAAGACGATAGTTACCGGATAAGGCG  
CAGCGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTAC  
ACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGA  
AAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTT  
CCAGGGGGAAACGCCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAG  
CGTCGATTTTTTGTGATGCTCGTCAGGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCG  
GCCTTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTA  
TCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGC-

AGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGC  
AAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTT  
TTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTGAA  
TGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCACCT  
GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGG  
CCCTTTCACCTCATTAG

FIGURE 91D

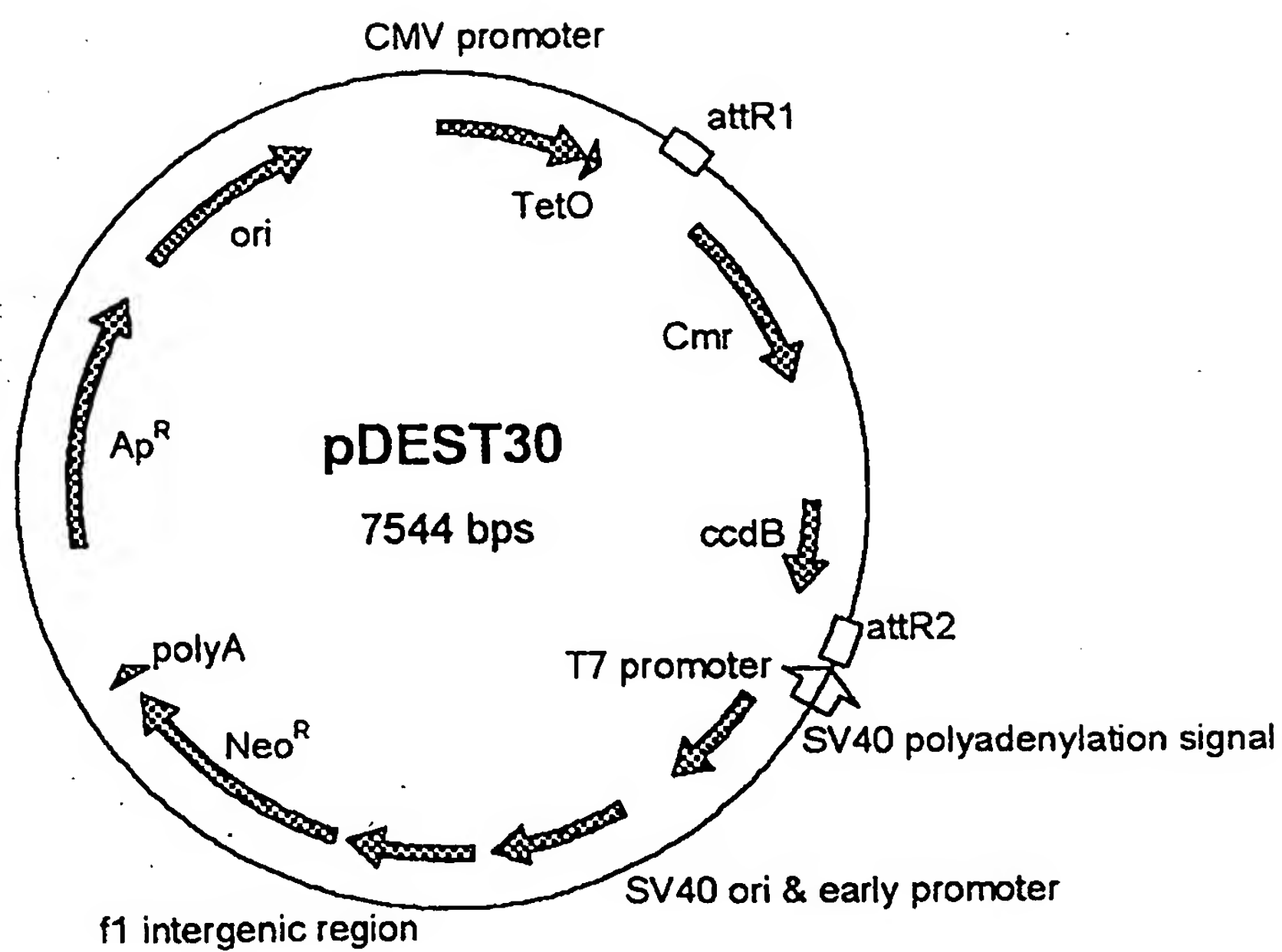


FIGURE 92A



pDEST30

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ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC  
CGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT  
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGAC  
TCACGGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACT  
CTAGAGGATCCCTACCGGTGATATCCTCGAGCCCATCAACAAGTTTGTACAAAAAAGCTG  
AACGAGAAACGTAAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGGCGGCCGCATTAGGCAC  
CCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGAGTTAGGATCC  
GGCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCAC  
CGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCA  
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AAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCTCA  
TCCGGAATTCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCC  
TTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTTTCATCGCTCTGGAGTGAATACCA  
CGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAA  
CCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTG  
GGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGT  
TTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCA  
GGTTCATCATGCCGTCTGTGATGGCTTCCATGTGCGCAGAATGCTTAATGAATTACAACA  
GTACTGCGATGAGTGGCAGGGCGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAG  
ATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTA  
TACCCGAAGTATGTCAAAAAGAGGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGAC  
AGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCA  
CAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGG  
AAGGGATGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACA  
GGGACTGGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTG  
TTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTG  
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GGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATC  
GGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTG  
ATGTTCTGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCA  
TAGTGAAGTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTA  
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TCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGTTTTACAACGTCGTGA  
CTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATA  
ATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTTAAGTGT  
ATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTTGCTTACTGAGTATGA  
TTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTGTGTATTTTAGATTCA  
CAGTCCCAAGGCTCATTTTCAGGCCCTCAGTCCTCACAGTCTGTTTCATGATCATAATCAG  
CCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAA  
CCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGG  
TTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTTC  
TAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCGATCCTGCATT  
AATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTTGCGTATTGGCTGGCGTAATAGCGAAG  
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CCTGTAGCGGCGCATTAAAGCGCGGGGTTGGTGGTTACGCGCAGCGTGACCGCTACAC  
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CCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT-

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CCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT  
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TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAATATTTAACGCGA  
ATTTTAACAAATATTTAACGTTTACAATTTTCGCCTGATGCGGTATTTTCTCCTTACGCAT  
CTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCATGGCCTGAAATAACCT  
CTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCGGAAAGAACCAGCTGTGGAATGTGT  
GTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGC  
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GGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGC  
CGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGG  
TGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGT  
TCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGG  
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CATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCA  
CCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCA  
GGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCCAGGCTCAA  
GGCGCGCATGCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAA  
TATCATGGTGGAAAATGGCCGCTTTTCTGGATTCACTGACTGTGGCCGGCTGGGTGTGGC  
GGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGA  
ATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGATTCCGACGCGCATCGC  
CTTCTATCGCCTTCTTGACGAGTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGAC  
CAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATATCTTTATTTTCATTACA  
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CTCCGGGAGCTGCATGTGTGTCAGAGGTTTTACCCTCATCACCGAAACGCGCGAGACGAAA  
GGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGAC  
GTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAAT  
ACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATATTG  
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TCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA  
GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGG  
CGCGGTATTATCCCGTATTGACGCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTC  
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AGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAAGTGCAGGCAACTTACT  
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TGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCG  
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CGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGC  
TGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTGACACCAAGTTTACTCATATAT  
ACTTTAGATTGATTTAAACTTCAATTTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTT  
TGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCC  
CGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTT  
GCAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCGGATCAAGAGCTACCAAC  
TCTTTTTCCGAAGGTAAGTGGCTTACGACAGCGCAGATAACCAATACTGTCCTTCTAGT  
GTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCT  
GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGA  
CTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCTGTCAC-



ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTG  
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TGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG  
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CTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAG  
CGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCA  
TTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAGCATTATCAGGGTTA  
TTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAATAGGGGTTCC  
GCGCACATTTCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTTATTATCATGACATT  
AACCTATAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTAG

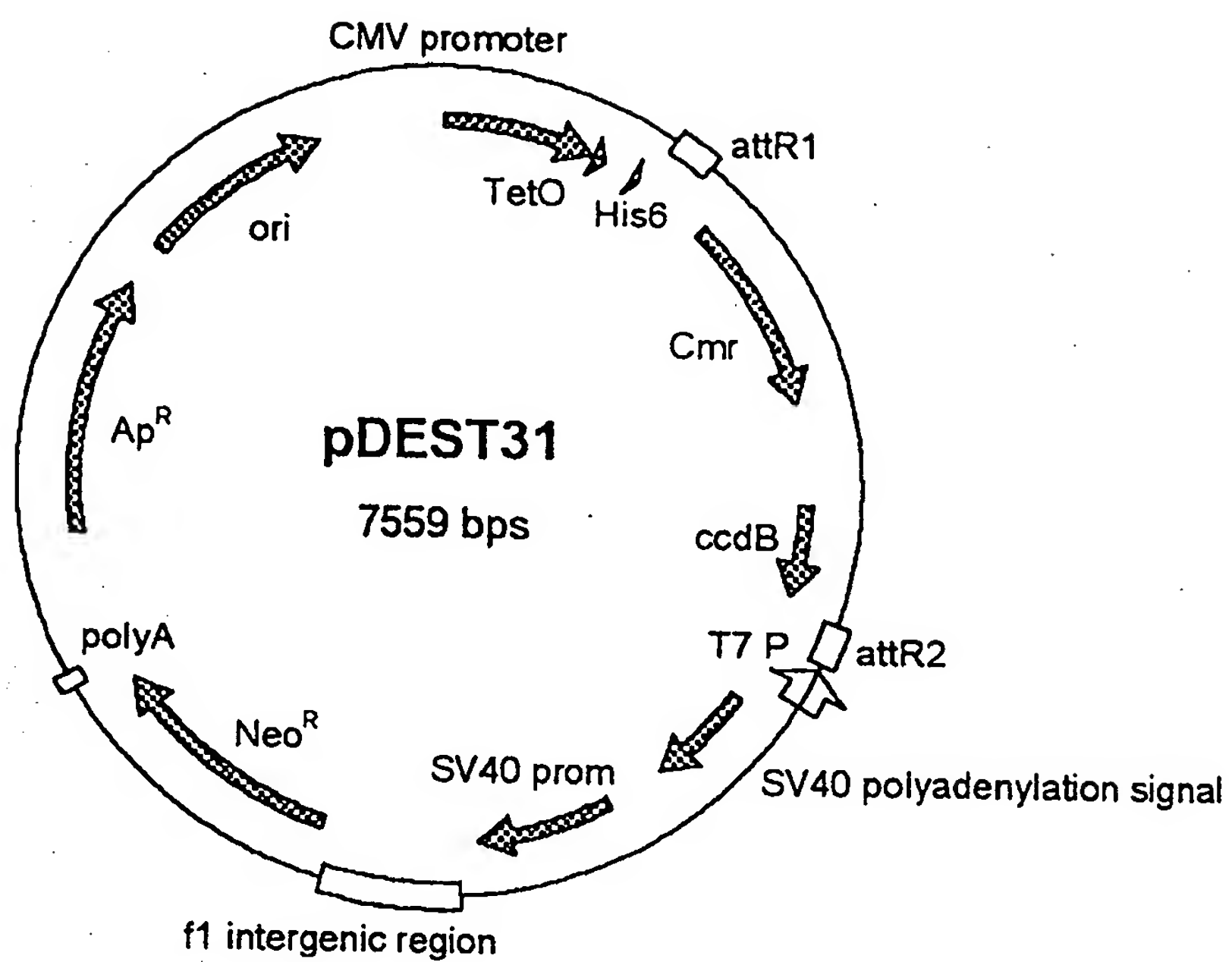


FIGURE 93A

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pDEST31 7559 bp

ATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCC  
CGCCCATGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT  
TGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT  
CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT  
GCCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATC  
GCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGAC  
TCACGGGGATTTCCAAGTCTCCACCCCATGACGTCAATGGGAGTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCTGTAACAACCTCCGCCCCATTGACGCAAATGGGCGGT  
AGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCCCTATCAGTGATAGAGATCTC  
CCTATCAGTGATAGAGATCGTCGACGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGA  
CGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGGACC  
ATGGCGTACTACCATCACCATCACCATCACACCGGTGATATCCTCGAGCCCATCACAAAGT  
TTGTACAAAAAAGCTGAACGAGAAACGTAAATGATATAAATATCAATATATTAAATTAG  
ATTTTGCATAAAAAACAGACTACATAATACTGTAAACACACATATCCAGTCACTATGG  
CGGCCGATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGA  
TTTTGAGTTAGGATCCGGCGAGATTTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAA  
TCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCAT  
TTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTT  
TAAAGACCGTAAAGAAAAATAAGCACAAAGTTTATCCGGCCTTTATTACATTCTTGCCC  
GCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGATAT  
GGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGC  
TCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTGCAAGATGTGG  
CGTGTTACGGTGAAAACCTGGCCTATTTCCCTAAAGGGTTTTATTGAGAATATGTTTTTCG  
TCTCAGCCAATCCCTGGGTGAGTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACA  
ACTTCTTCGCCCCCGTTTTTACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGA  
TGCCGCTGGCGATTACAGGTTTCATCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGC  
TTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAACGCGTGGATCCG  
GCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAA  
TATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTAT  
TACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATC  
TCCGGTCTGGTAAGCACAAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGG  
AAAGCGGAAAATCAGGAAGGGATGGCTGAGGTGCGCCCGTTTTATTGAAATGAACGGCTCT  
TTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGGTTTACACCTATAAAAGAGA  
GAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCCGGGCGACG  
GATGGTGATCCCCCTGGCCAGTGACAGTCTGCTGTGATGATAAAGTCTCCCGTGAACCTTA  
CCCGGTGGTGATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGT  
GCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAA  
AAACGCCATTAACTGATGTTCTGGGGAATATAAATGTGAGGCTCCGTTATACACAGCCA  
GTCTGCAGGTGACCATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTT  
TTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTTCAG  
CTTTCTTGTAACAAGTGGTGATGGGCGGCCGCTCTAGAGGGCCCAAGCTTACGCGTGAT  
GCGACGTCATAGCTCTCTCCCTATAGTGAGTCGTATTATAAGCTAGGCACTGGCCGTCGT  
TTTACAACGTCGTGACTGGGAAAACCTGCTAGCTTGGGATCTTTGTGAAGGAACCTTACTT  
CTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATAT  
AAAATTTTTAAGTGTATAATGTGTTAAACTAGCTGCATATGCTTGCTGCTTGAGAGTTTT  
GCTTACTGAGTATGATTTATGAAAATATTATACACAGGAGCTAGTGATTCTAATTGTTTG  
TGATTTTTAGATTCACAGTCCCAAGGCTCATTTACGGCCCCCTCAGTCCTCACAGTCTGTT  
CATGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCC  
ACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTAT  
TGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCAAATTTACAAATAAAGCATT  
TTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTG  
GATCGATCCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTGGCT  
GGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATG  
GCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGGGTTGGTGGTTACGCGCA  
GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCT  
TTCTCGCCACGTTCCCGGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGT-

Figure 93B

TCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCAC  
GTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCT  
TTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTT  
TTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC  
AAATATTTAACGCGAATTTTAACAAAATATTAAACGTTTACAATTTTCGCTGATGCGGTAT  
TTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATACGCGGATCTGCGCAGCACCAT  
GGCCTGAAATAACCTCTGAAAGAGGAACCTTGGTTAGGTACCTTCTGAGGCGGAAAGAACC  
AGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAA  
GTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCC  
CAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCC  
TAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCATTTCTCCGCCCATGGCT  
GACTAATTTTTTTTTATTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGA  
AGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTTGATTCTTCTGACA  
CAACAGTCTCGAACTTAAGGCTAGAGCCACCATGATTGAACAAGATGGATTGCACGCAGG  
TTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGG  
CTGCTCTGATGCCGCCGTGTTCCGGCTGTGACGCGAGGGGCGCCCGGTTCTTTTTGTCAA  
GACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCT  
GGCCACGACGGGCGTTCTTGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGA  
CTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCTCATCTCACCTTGCTCCTGC  
CGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTAC  
CTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGC  
CGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACT  
GTTCCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGA  
TGCCTGCTTGCCGAATATCATGGTGGAATGGCCGCTTTTCTGGATTCTCGACTGTGG  
CCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGA  
AGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGA  
TTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGG  
TTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATATC  
TTTATTTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGATAGCGATAAGGATCCG  
CGTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACA  
CCCCGCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAG  
ACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGTCAGAGGTTTTTCACCGTCATCACCGAA  
ACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAAT  
AATGGTTTTCTTAGACGTGAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTG  
TTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT  
GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCCGCCCTTAT  
TCCCTTTTTTGCGGCATTTTGCCTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGT  
AAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAG  
CGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAA  
AGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCG  
CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT  
TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACAC  
TGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA  
CAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT  
ACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT  
ATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGC  
GGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTTATTGCTGA  
TAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGG  
TAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACG  
AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAAGCATTGGTAACTGTGAGACCA  
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GGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCTGTTCCA  
CTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG  
CGTAATCTGCTGCTTGCAACAAAAAAACCACCGCTACCAGCGGTGGTTTTGTTTGCCGGA  
TCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAA  
TACTGTCCTTCTAGTGAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCC  
TACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTG  
TCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGAAC-

GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCT  
ACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCC  
GGTAAGCGGCAGGGTCCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTG  
GTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATG  
CTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCT  
GGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGA  
TAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCG  
CAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGC  
GCGTTGGCCGATTCATTAATGCAGAGCTTGCAATTCGCGCGTTTTTCAATATTATTGAAG  
CATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAA  
ACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCAT  
TATTATCATGACATTAACCTATAAAAATAGGCGTAGTACGAGGCCCTTCACTCATTAG

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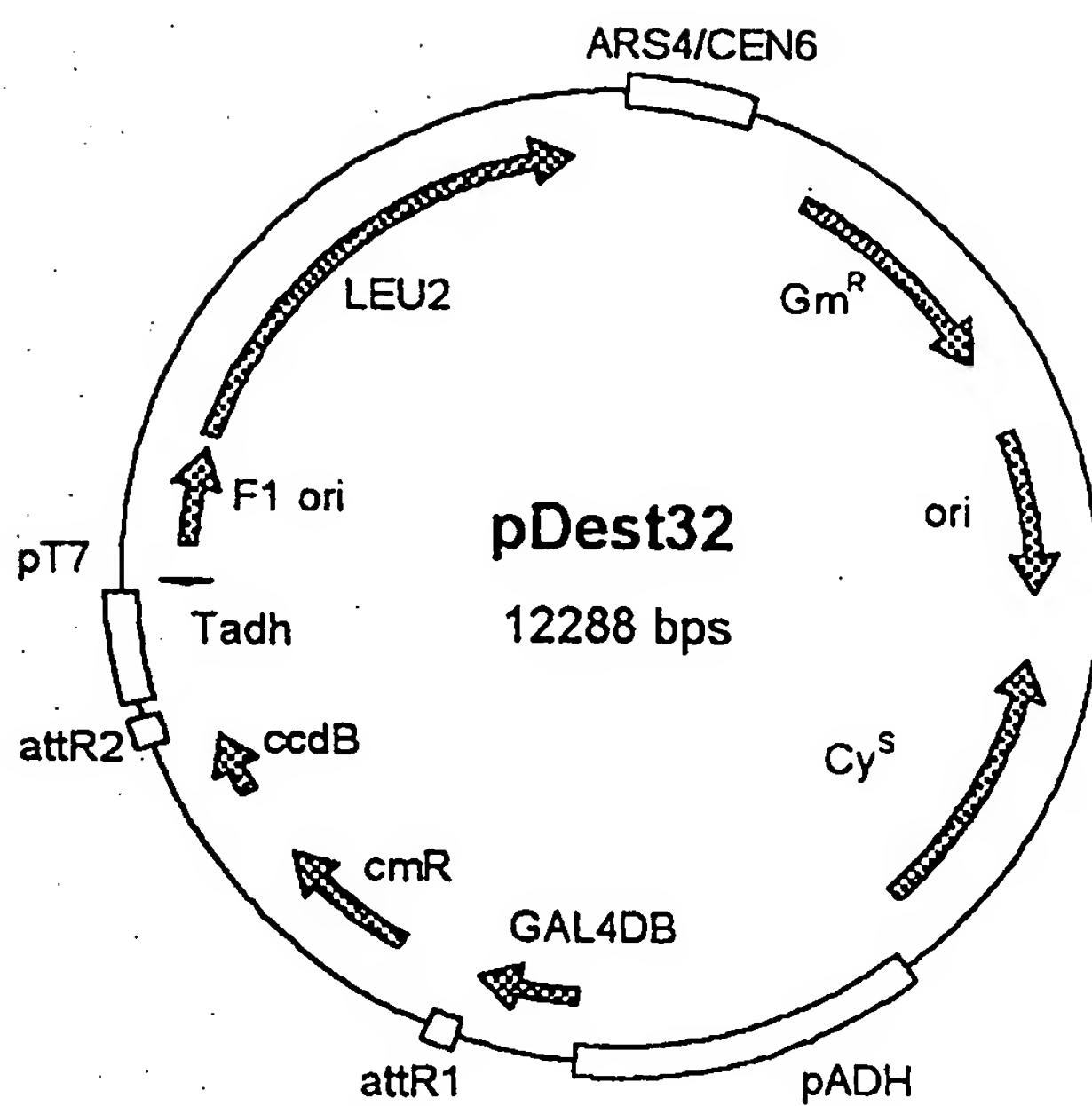


FIGURE 94A



pDEST32 12288 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT  
CTTAGGACGGATCGCTTGCCTGTAACCTTACACGCGCCTCGTATCTTTTAATGATGGAATA  
ATTTGGGAATTTACTCTGTGTTTATTTATTTTTATGTTTTGTATTTGGATTTTAGAAAGT  
AAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAAATAAACAAAGGTTTAAAAA  
ATTTCAACAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATA  
GATATACATTTCGATTAAACGATAAGTAAATGTAAATCACAGGATTTTCGTGTGTGGTCT  
TCTACACAGACAAGATGAAACAATTCGGCATTAAATACCTGAGAGCAGGAAGAGCAAGATA  
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAACAAAACT  
ATTTTTTCTTTAATTTCTTTTTTTTACTTTCTATTTTTTAATTTATATATTTATATTA  
ATTTAAATTATAATTATTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG  
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCG  
CTCATGAGACAATAACCCCTGATAAATGCTTCAATAATCTGCAGTGCAGGCGCCGTGTC  
TCAAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACAATAAACT  
GTCTGCTTACATAAACAGTAATAACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTC  
TTGCTGGAGGCGCGATTAAATTCACATGATGCTGATTTATATGGGTATAAATGGGC  
TCGGTAGCCAACCACTAGAACTATAGCTAGAGTCTTGGGCGAACAAACGATGCTCGCCTT  
CCAGAAAACCGAGGATGCGAACCCTTCAATCCGGGGTCAGCACCACCGCAAGCGCCGCG  
ACGGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGCAGATCCGTGCACAGCACCTTGCCGT  
AGAAGAACAGCAAGGCCGCAATGCCTGACGATGCGTGGAGACCGAAACCTTGCGCTCGT  
TCGCCAGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCAAGGTTGCCGGGTGACGCA  
CACCGTGGAACGGATGAAGGCACGAACCCAGTTGACATAAGCCTGTTCCGTTTCGTAAAC  
TGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAACGCAGCG  
GTGGTAACGGCGCAGTGCGGTTTTTCATGGCTTGTTATGACTGTTTTTTGTACAGTCTA  
TGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTTCGATGTTTGATGTTATGGA  
GCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGCAGGGCAGTCGCCCTAAAACA  
AAGTTAGGTGGCTCAAGTATGGGCATCATTTCGCACATGTAGGCTCGGCCCTGACCAAGTC  
AAATCCATGCGGGCTGCTCTTGATCTTTTCGGTTCGTGAGTTTCGGAGACGTAGCCACCTAC  
TCCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTTGCTCCGTAGTAAGACATTCATC  
GCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTCGCGGCTTACGTTCTGCCC  
AGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTCGCAGTCTCCGGCGAGCAC  
CGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAGCATGAGGCCAACGCGCTT  
GGTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGATCCCGCAGTGGCTCTCTAT  
ACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATCGACCCAAGTACCGCCACC  
TAACAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCCTAATAGGTTGTATTGATGTTGGAC  
GAGTCGGAATCGCAGACCGGATACAGGATCTTGCCATCCTATGGAACCTGCCTCGGTGAGT  
TTTCTCCTTCATTACAGAAACGGCTTTTTTCAAAAATATGGTATTGATAATCCTGATATGA  
ATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGT  
TGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGNCATGACCAAAATCCCTT  
AACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT  
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACACCGCTACCAG  
CGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCA  
GCAGAGCGCAGATACCAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA  
AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG  
CCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCAGGATAAGG  
CGCAGCGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGAGAGCGAACGACCT  
ACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGA  
GAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGC  
TTCCAGGGGGGAACGCTTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTG  
AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGAGCCTATGGAAAACGCCAGCAACG  
CGGCCTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGT  
TATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC  
GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATAC  
GCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCAGCAGAGGTTTC  
CCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACTCATTAGG  
CACCCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTGAGCGGAT  
AACAAATTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATTAACCTC-

Figure 94B

ACTAAAGGGAACAAAAGCTGGTACCGATCCCGAGCTTTGCAAATTAAAGCCTTCGAGCGT  
CCCAAAACCTTCTCAAGCAAGGTTTTTCAGTATAATGTTACATGCGTACACGCGTCTGTAC  
AGAAAAAAAGAAAAATTTGAAATATAAATAACGTTCTTAATACTAACATAACTATAAAA  
AAATAAATAGGGACCTAGACTTCAGGTTGTCTAACTCCTTCCTTTTCGGTTAGAGCGGAT  
GTGGGGGGAGGGCGTGAATGTAAGCGTGACATAACTAATTACATGATATCGACAAAGGAA  
AAGGGGCTGTTTACTCACAGGCTTTTTTCAAGTAGGTAATTAAGTCGTTTCTGTCTTTT  
TCCTTCTTCAACCCACCAAAGGCCATCTTGGTACTTTTTTTTTTTTTTTTTTTTTTTTTT  
TT  
TTTTTTTTTCATAGAAATAATACAGAAGTAGATGTTGAATTAGATTAAACTGAAGATATAT  
AATTTATTGGAATAACATAGAGCTTTTTGTTGATGCGCTTAAGCGATCAATTCACAAC  
ACCACCAGCAGCTCTGATTTTTTCTTCAGCCAACCTTGGAGACGAATCTAGCTTTGACGAT  
AACTGGAACATTTGGAATTCTACCTTACCCAAGATCTTACCGTAACCGGCTGCCAAAGT  
GTCAATAACTGGAGCAGTTTCCTTAGAAGCAGATTTCAAGTATTGGTCTCTCTTGTCTTC  
TGGGATCAATGTCCACAATTTGTCCAAGTTCAAGACTGGCTTCCAGAAATGAGCTTGTTG  
CTTGTGGAAGTATCTCATACCAACCTTACCGAAATAACCTGGATGGTATTTATCCATGTT  
AATCTGTGGTGATGTTGACCACCGGCCATACCTCTACCACCGGGGTGCTTTCTGTGCTT  
ACCGATACGACCTTTACCGGCTGAGACGTGACCTCTGTGCTTTCTAGTCTTAGTGAATCT  
GGAAGGCATTCTTGATTAGTTGGATGATTGTTCTGGGATTTAATGCAAAAATCACTTAAG  
AAGGAAAATCAACGGAGAAAGCAAACGCCATCTTAAATATACGGGATACAGATGAAAGGG  
TTTGAACCTATCTGGAATAAGCATTAACAAGCGAAAAACTGCGAGGAAAATGTTTGC  
GTCTCTGCGGGCTATTACGCGCCAGAGGAAAATAGGAAAAATAACAGGGCATTAGAAAA  
ATAATTTTGATTTTGGAATGTGTGGGTCTGGTGACAGATGTTACATTGGTTACAGTA  
CTCTTGTTTTTGCTGTGTTTTTCGATGAATCTCCAAAATGGTTGTTAGCACATGGAAGAG  
TCACCGATGCTAAGTTATCTCTATGTAAGCTACGTGGCGTGACTTTTGATGAAGCCGCAC  
AAGAGATACAGGATTGGCAACTGCAAATAGAATCTGGGGATCCCCCTCGAGATCCGGGA  
TCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATA  
TAAGGGTCGAACGAAAAATAAAGTGAAAAGTGTTGATATGATGTATTTGGCTTTGCGGCG  
CCGAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTC  
TTGCCGGCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGGCGGAGTTTTTTGCGCCTG  
CATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGG  
TTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCAATTATTTAAGTTGCCGAAAGAA  
CCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCAAGACTTGCGAGACGCGA  
GTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACC  
GCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTA  
CATACAACACTGGAAATGGTTGTCTGTTTGAGTACGCTTTCAATTCATTTGGGTGTGCAC  
TTTATTATGTTACAATATGGAAGGGAACTTTACACTTCTCCTATGCACATATATTAATTA  
AAGTCCAATGCTAGTAGAGAAGGGGGGTAACACCCCTCCGCGCTCTTTTCCGATTTTTTT  
CTAAACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTC  
CTCTTTTCTGGCAACCAAACCCATACATCGGGATTCTTATAATACCTTCGTTGGTCTCCC  
TAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATG  
GGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTGGTACATAACGAACTAAT  
ACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATT  
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TCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAAATGATGGAAGACACTAA  
AGGAAAAAATTAACGACAAAGACAGCAACAGATGTCGTTGTTCCAGAGCTGATGAGG  
GGTATCTTCGAACACACGAAACTTTTTCTTCTTCAATTCACGCACACTACTCTCTAATG  
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TCGTTCCCTTTCTTCTTGTCTTTTCTTGACAAATATTTCAAGCTATACCAAGCATAAC  
AATCAACTCCAAGCTTGAAGCAAGCCTCCTGAAAGATGAAGCTACTGTCTTCTATCGAAC  
AAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGTGCTCCAAAGAAAAACCGAAGTGCG  
CCAAGTGCTGAAGAACAACCTGGGAGTGTGCTACTCTCCCAAACCAAAGGTCTCCGC  
TGACTAGGGCACATCTGACAGAAGTGGAATCAAGGCTAGAAAGACTGGAACAGCTATTTT  
TACTGATTTTTCTCGAGAAGACCTTGACATGATTTTGAAATGGATTCTTTACAGGATA  
TAAAAGCATTGTTAACAGGATTATTTGTACAAGATAATGTGAATAAAGATGCCGTCACAG  
ATAGATTGGCTTCAGTGGAGACTGATATGCCTCTAACATTGAGACAGCATAGAATAAGTG  
CGACATCATCATCGGAAGAGAGTAGTAACAAAGGTCAAAGACAGTTGACTGTATCGTCTGA  
GGTCGAATCAAACAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATA-

TCAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAAACACAAC  
ATATCCAGTCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGA  
ATAAATACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGA  
TACCGGGAAGCCCTGGGCCAATTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGG  
TTCCAACCTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGTAGTTATCGAG  
ATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATAACCACGTTGA  
TATATCCCAATGGCATCGTAAAGAACATTTTGGAGGCATTTTCAGTCAGTTGCTCAATGTAC  
CTATAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTTTAAAGACCGTAAAGAAAAATAA  
GCACAAGTTTTATCCGGCCTTTATTCACATTCCTTGCCCGCCTGATGAATGCTCATCCGGA  
ATTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCAACCCTTGTTA  
CACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGA  
TTTCCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAACCTGGC  
CTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAG  
TTTCACCAGTTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTTAC  
CATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTCA  
TCATGCCGTCTGTGATGGCTTCCATGTCCGCAGAATGCTTAATGAATTACAACAGTACTG  
CGATGAGTGGCAGGGCGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACA  
GTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCG  
AAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGAC  
AGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCA  
TGCAGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGA  
TGGCTGAGGTGCCCCGGTTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACT  
GGTGAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTG  
GATGTACAGAGTGATATTATTGACACGCCCCGGCGACGGATGGTGATCCCCCTGGCCAGT  
GCACGTCTGCTGTGATATAAAGTCTCCCGTGAACCTTACCCGGTGGTGATATCGGGGAT  
GAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAA  
GAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTTC  
TGGGGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGACCATAGTGA  
CTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAA  
TATATTGATATTTATATCATTTTACGTTTTCTCGTTTCAGCTTTCTTGTACAAAGTGGTTTG  
ATGGCCGCTAAGTAAGTAAGACGTCGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTGG  
AGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTGCGCTTGTC  
TACCTTGCCAGAAATTTACGAAAAGATGGAAAAGGGTCAAATCGTTGGTAGATACGTTGT  
TGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTAT  
AAAAAAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAAACGAAAATTTCTT  
GTTCTTGAGTAACTCTTTCCTGTAGGTGAGGTTGCTTTCTCAGGTATAGCATGAGGTCGC  
TCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATTT  
CACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTTA  
TGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTCGCCCTA  
TAGTGAGTCGTATTACAATTCAGTGGCCGTGTTTTACAACGTCGTGACTGGGAAAACCC  
TGGCGTTACCCAACCTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAG  
CGAAGAGGCCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGAC  
GCGCCCTGTAGCGGCGCATTAAGCGCGGGCGGTGTGGTGGTTACGCGCAGCGTGACCGCT  
ACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACG  
TTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGT  
GCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCA  
TCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA  
CTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAA  
GGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAAC  
GCGAATTTTAAACAAAATATTAACGTTTACAATTTCTGATGCGGTATTTTCTCCTTACGC  
ATCTGTGCGGTATTTACACCGCATATCGACCGGTGAGGAGAACTTCTAGTATATCCAC  
ATACCTAATATTATTGCCTTATTAAAAATGGAATCGGAACAATTACATCAAAATCCACAT  
TCTCTTCAAAATCAATTGTCTGTACTTCTTGTTCATGTGTGTTCAAAAACGTTATATT  
TATAGGATAATTATACTCTATTTCTCAACAAGTAATTGGTTGTTTGGCCGAGCGGTCTAA  
GGCGCTGATTCAAGAAATATCTTGACCGCAGTTAACTGTGGGAATACTCAGGTATCGTA  
AGATGCAAGAGTTTGAATCTCTTAGCAACCATTATTTTTTTCTCAACATAACGAGAACA  
CACAGGGGCGCTATCGCACAGAATCAAATTCGATGACTGGAAATTTTTTGTTAATTTTCA  
AGGTCGCCTGACGCATATACCTTTTTCAACTGAAAATTTGGGAGAAAAGGAAAGGTGAG-



AGGCCGGAACCGGCTTTTCATATAGAATAGAGAAGCGTTCATGACTAAATGCTTGCATCA  
CAATACTTGAAGTTGACAATATTATTTAAGGACCTATTGTTTTTTCCAATAGGTGGTTAG  
CAATCGTCTTACTTTCTAACTTTTCTTACCTTTTACATTTTACGCAATATATATATATATT  
TCAAGGATATACCATTCTAATGTCTGCCCCCTATGTCTGCCCCCTAAGAAGATCGTCGTTTT  
GCCAGGTGACCACGTTGGTCAAGAAATCACAGCCGAAGCCATTAAGGTTCTTAAAGCTAT  
TTCTGATGTTTCGTTCCAATGTCAAGTTCGATTTTCGAAAATCATTTAATTGGTGGTGCTGC  
TATCGATGCTACAGGTGTCCCACTTCCAGATGAGGCGCTGGAAGCCTCCAAGAAGGTTGA  
TGCCGTTTTGTTAGGTGCTGTGGGTGGTCCTAAATGGGGTACCGGTAGTGTAGACCTGA  
ACAAGGTTTACTAAAAATCCGTAAAGAACTTCAATTGTACGCCAACTTAAGACCATGTAA  
CTTTGCATCCGACTCTCTTTTAGACTTATCTCCAATCAAGCCACAATTTGCTAAAGGTAC  
TGACTTCGTTGTTGTCAGAGAATTAGTGGGAGGTATTTACTTTGGTAAGAGAAAGGAAGA  
CGATGGTGATGGTGTCTGCTTGGGATAGTGAACAATACACCGTTCCAGAAGTGCAAAGAAT  
CACAAGAATGGCCGCTTTTCATGGCCCTACAACATGAGCCACCATTGCCTATTTGGTCCTT  
GGATAAAGCTAATGTTTTGGCCTCTTCAAGATTATGGAGAAAACTGTGGAGGAAACCAT  
CAAGAACGAATTCCTACATTGAAGGTTCAACATCAATTGATTGATTCTGCCGCCATGAT  
CCTAGTTAAGAACCCAACCCACCTAAATGGTATTATAATCACCAGCAACATGTTTGGTGA  
TATCATCTCCGATGAAGCCTCCGTTATCCCAGGTTCTTGGGTTTGTGTCATCTGCGTC  
CTTGGCCTCTTTGCCAGACAAGAACACCGCATTTGGTGTGTACGAACCATGCCACGGTTC  
TGCTCCAGATTTGCCAAAGAATAAGGTTGACCCTATCGCCACTATCTGTCTGCTGCAAT  
GATGTTGAAATTGTCATTGAACTTGCTGAAGAAGGTAAGGCCATTGAAGATGCAGTTAA  
AAAGGTTTTGGATGCAGGTATCAGAACTGGTGATTAGGTGGTTCCAACAGTACCACCGA  
AGTCGGTGATGCTGTCCCGAAGAAGTTAAGAAAATCCTTGCTTAAAAAGATTCTCTTTT  
TTTATGATATTTGTACATAAACTTTATAAATGAAATTCATAATAGAAACGACACGAAATT  
ACAAAATGGAATATGTTTCATAGGGTAGACGAACTATATACGCAATCTACATACATTTAT  
CAAGAAGGAGAAAAAGGAGGATAGTAAAGGAATACAGGTAAGCAAATTGATACTAATGGC  
TCAACGTGATAAGGAAAAAGAATTGCACCTTTAACATTAATATTGACAAGGAGGAGGGCAC  
CACACAAAAGTTAGGTGTAACAGAAAATCATGAACTACGATTCTAATTTGATATTGG  
AGGATTTTCTCTAAAAAATAACAATAAAAAACACTCAATGACCTGACCAT  
TTGATGGAGTTTAAGTCAATACCTTCTTGAACCATTTCCCATATGGTGAAAGTTCCCTC  
AAGAATTTTACTCTGTGAGAAACGGCTTACGACGTAGTCGATATGGTGCACTCTCAGTA  
CAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACG  
CGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCG  
GGAGCTGCATGTGTGTCAGAGGTTTTACCGTCATACCGAAACGCGCGA

FIGURE 94E

222/240

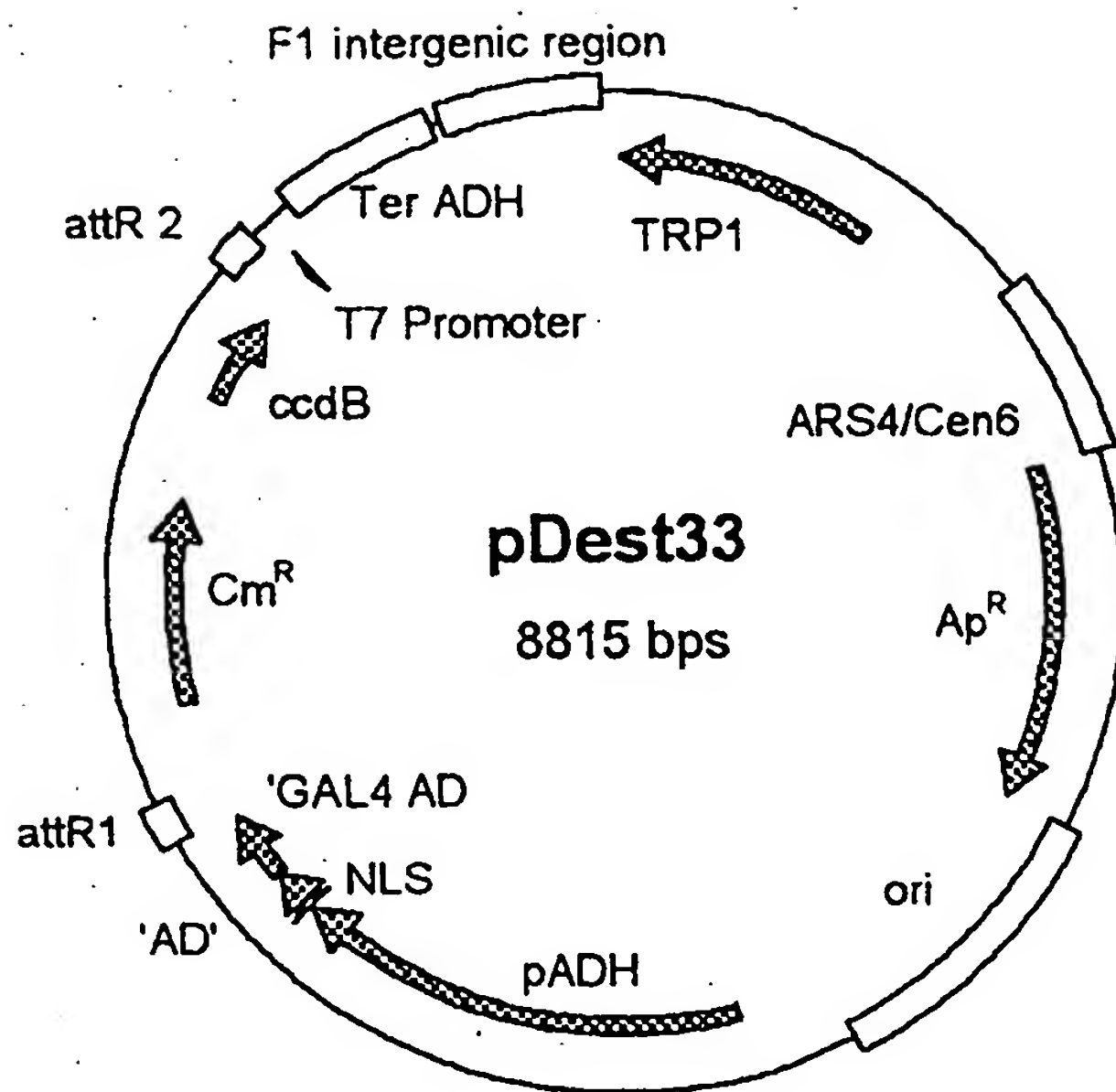


FIGURE 95A

pDEST33 8815 bp

GCCTTACGCATCTGTGCGGTATTTACACCCGAGGCAAGTGCACAAACAATACTTAAATA  
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTTGACGAAATTTGCTATTTTGTTAG  
AGTCTTTTACACCATTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA  
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC  
TTTCGGGGCTCTCTTGCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCAC  
CTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG  
CACTGAGTAGTATGTTGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGA  
GGAACCTTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT  
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT  
ATTTTCGGAGTGCCTGAACTATTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAA  
TAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCAGCAAGTCAGCAT  
CGGAATCTAGAGCACATTCTGCGGCTCTGTGCTCTGCAAGCCGCAAACTTTCACCAATG  
GACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA  
TCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTC  
TTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAGAAAGCTCCGGATCAAGATTGT  
ACGTAAGGTGACAAGCTATTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC  
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATA  
TATAGTAATGTCGTTTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA  
GCCAGCCCCGACACCCGCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG  
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTCAC  
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTATAGGTTA  
ATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCCCTGTAACCTACACGCGCCTC  
GTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT  
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA  
AAATAAACAAAGGTTTAAAAAATTTCAACAAAAGCGTACTTTACATATATATTTATTAG  
ACAAGAAAAGCAGATTAAATAGATATACATTTCGATTAACGATAAGTAAATGTAAATCA  
CAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAATACCT  
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA  
CATCTTCGGAAAACAAAACCTATTTTCTTTAATTTCTTTTTTTACTTTCTATTTTAA  
TTTATATATTTATATTAATAAATTTAAATTATAATTATTTTATAGCACGTGATGAAAAG  
GACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAA  
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATAT  
TGAAAAGGAAGAGTATGAGTATTTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTGCG  
GCATTTTGCTTCTGTTTGTCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAA  
GATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTT  
GAGAGTTTTCGCCCCGAAGAACGTTTCCCAATGATGAGCACTTTTAAAGTTCTGCTATGT  
GGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTAT  
TCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCCTTTTTTTCACAACATGGGGGAT  
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAAGTGGCGAA  
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCC  
GGTGAGCGTGGGTCTCGCGGTATCATTTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC  
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAAGTGTGAGACCAAGTTTACTCATAT  
ATACTTTAGATTGATTTAAACCTTCATTTTAAATTTAAAGGATCTAGGTGAAGATCCTT  
TTTGATAATCTCATGACCAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGAC  
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGC  
TTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA  
ACTCTTTTCCGAAGGTAAGTGGCTTCAAGAGCGCAGATAACCAATACTGTCCTTCTA  
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT  
CTGCTAATCCTGTTACAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG  
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCTGTGC  
ACACAGCCCAGCTTGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT-

FIGURE 95B



TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG  
GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT  
CCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGG  
CCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGG  
CCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC  
GCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAGTG  
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATT  
CATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA  
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT  
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT  
GATTACGCCAAGCTCGGAATTAACCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC  
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG  
AAGGCAAAAGACAAATATAAGGGTCAACGAAAAATAAAGTGAAAAGTGTTGATATGATG  
TATTTGGCTTTGCGGCGCCGAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCT  
GTGGCGGACCCGCGCTCTTGCCGGCCCGCGGATAACGCTGGGCGTGAGGCTGTGCCCGGC  
GGAGTTTTTTGCGCCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA  
AGCAATAAGAATGCCGGTTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCAATTAT  
TTAAGTTGCCGAAAGAACCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCA  
AGACTTGCGAGACGCGAGTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAG  
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA  
GTATAAATAGACAGGTACATAACAACCTGGAAATGGTTGTCTGTTTGAGTACGCTTTCAA  
TTCATTTGGGTGTGCACCTTTATTATGTTACAATATGGAAGGGAACCTTTACACTTCTCCTA  
TGCACATATATTAATTAAAGTCCAATGCTAGTAGAGAAGGGGGTAACACCCCTCCGCGC  
TCTTTTCCGATTTTTTTCTAAACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGG  
TGTACAATATGGACTTCCTCTTTTCTGGCAACCAAACCCATACATCGGGATTCCCTATAAT  
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATA  
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG  
GTACATAACGAACATAACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC  
ACTACCCTTTTCCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC  
TTTTTTTTTCTTTCTCTCTCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAA  
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTG  
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTTCCTTCCTTCATTACG  
CACACTACTCTCTAATGAGCAACGGTATACGGCCTTCCTTCAGTTACTTGAATTTGAAA  
TAAAAAAAGTTTGCCGCTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG  
TTTCTCGTCATTGTTCTCGTTCCCTTTCTTCTTCTTTTCTGACAAATATTTCA  
AGCTATACCAAGCATAACAATCAACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCG  
AGCGGCGCCAATTTTAATCAAAGTGGAATATTGCTGATAGCTCATTGTCCTTCACTTTC  
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAAACAAATTCTCAAGCGCTTTC  
CAACCAATTGCCTCCTCTAACGTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGT  
AAAATTGATGATGGTAATAATTCAAACCACTGTACCTGGTTGGACGGACCAAACCTGCG  
TATAACGCGTTTGGAAATCACTACAGGGATGTTTAATACCACTACAATGGATGATGTATAT  
AACTATCTATTCGATGATGAAGATACCCACCAAACCCAAAAAAGAGGGTGGGTGCAAT  
CAAACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAATAATGATATAAATATCAATATA  
TTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACATATCCAG  
TCACTATGGCGGCCGCTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAATAAATAC  
CTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGA  
AGCCCTGGGCCAACTTTTGGCGAAATGAGACGTTGATCGGCACGTAAGAGGTTCCAACCT  
TTCACCATATGAAATAAGATCACTACCGGGCGTATTTTTTGTGATTATCGAGATTTTCAG  
GAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACACCGTTGATATATCCC  
AATGGCATCGTAAAGAACATTTTGGAGGCACTTCAGTCAGTTGCTCAATGTACCTATAACC  
AGACCGTTCAGCTGGATATTACGGCCTTTTAAAGACCGTAAAGAAAAATAAGCACAAAGT  
TTTATCCGCGCTTTATTACATTTCTTGCCCGCCTGATGAATGCTCATCCGGAATTCCGTA  
TGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTACCCCTTGTTACACCGTTT  
TCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGC  
AGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCC  
CTAAAGGGTTTATTGAGAATATGTTTTCTGCTCTCAGCCAATCCCTGGGTGAGTTTCACCA  
GTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTCGCCCCCGTTTTTACCATGGGCA  
AATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTACAGGTTTCATCATGCCG-

FIGURE 95C

TCTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGT  
GGCAGGGCGGGGCGTAATCTAGAGGATCCGGCTTACTAAAAGCCAGATAACAGTATGCGT  
ATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGT  
CAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCA  
GTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAAT  
GAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGGCTGAG  
GTCGCCCCGGTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAAT  
GCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACA  
GAGTGATATTATTGACACGCCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGACAGTCT  
GCTGTCAGATAAAGTCTCCCGTGAACTTTACCCGGTGGTGATATCGGGGATGAAAGCTG  
GCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGC  
TGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAAT  
ATAAATGTCAGGCTCCGTTATACACAGCCAGTCTGCAGGTCGACCATAGTGACTGGATAT  
GTTGTGTTTTTACAGTATTATGTAGTCTGTTTTTTTATGCAAAATCTAATTTAATATATTGA  
TATTTATATCATTTTACGTTTCTCGTTTCAGCTTTCTTGTAACAAGTGGTTTGATGGCCGC  
TAAGTAAGTAAGACGTCGAGCTCCCTATAGTGAGTCGTATTACACTGGCCGTCGTTTTAC  
AACGTCGTGACTGGGAAAACACCGGTGAGCTCTAAGTAAGTAACGGCCGCCACCGCGGTG  
GAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTCCGGCTTGT  
CTACCTTGCCAGAAATTTACGAAAAGATGGAAAAGGGTCAAATCGTTGGTAGATACGTTG  
TTGACACTTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTA  
TAAAAAAAATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAAACGAAAATTCT  
TGTTCTTGAGTAACTCTTTCCTGTAGGTGAGGTGCTTTCTCAGGTATAGCATGAGGTGCG  
CTCTTATTGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCCATT  
TCACCCAATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTT  
ATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCGCATCAGGCCGA  
AATTGTAAACGTTAATATTTTGTTAAATTCGCGTTAAATATTTGTTAAATCAGCTCATT  
TTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGAT  
AGGGTTGAGTGTTGTTCCAGTTTGGAAACAAGAGTCCACTATTAAAGAACGTGGACTCCAA  
CGTCAAAGGGCGAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCTTA  
ATCAAGTTTTTTGGGGTGGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCC  
CCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGC  
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGTAACCACCAC  
ACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCACTGCA

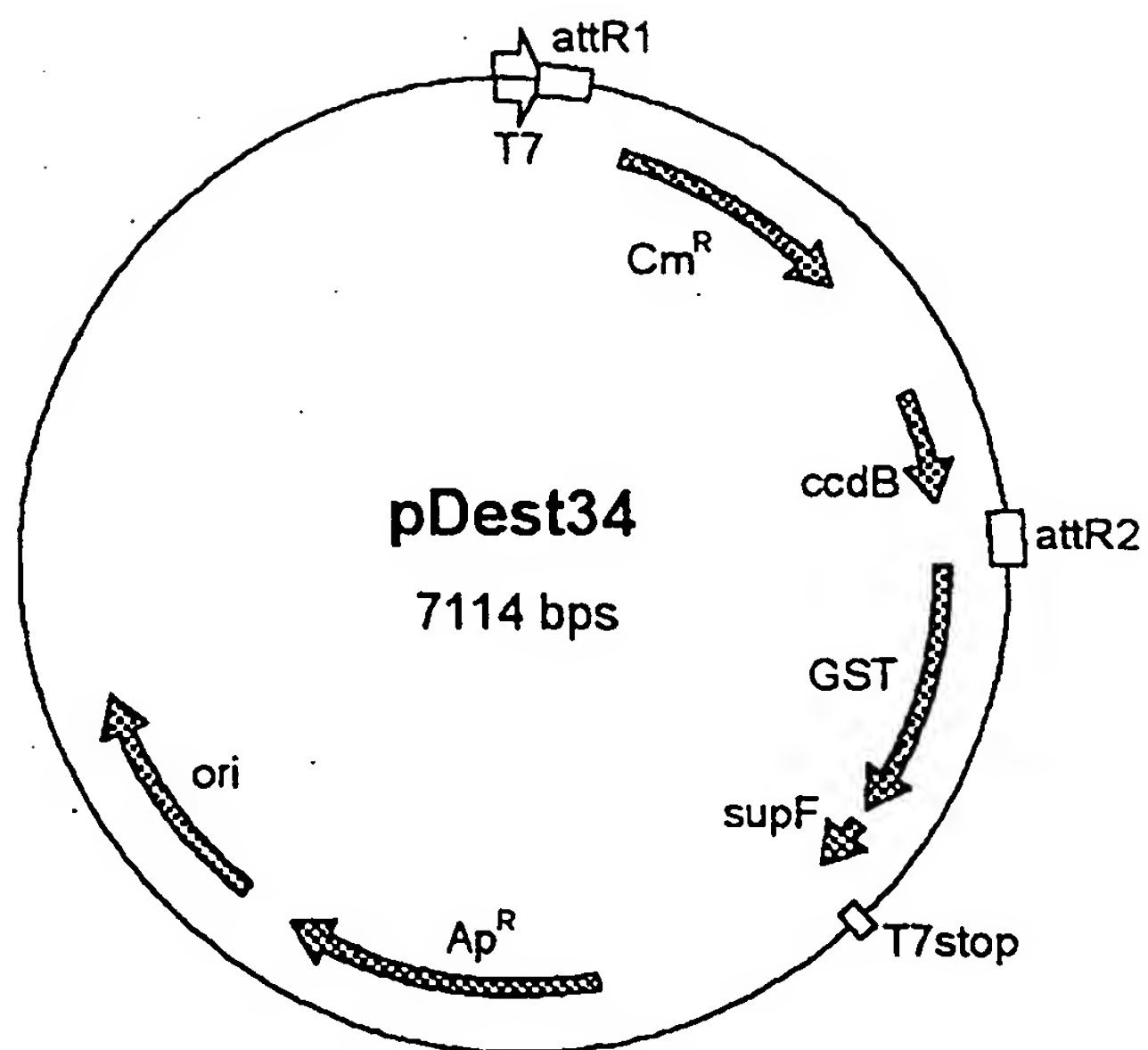


FIGURE 96A

pDEST34 7114 bp

<u>Location (Base Nos.)</u>	<u>Gene Encoded</u>
195..71	attR1
304..963	CmR
1305..1610	ccdB
1651..1775	attR2
1780..2472	GST
2675..2720	T7stop
3334..4194	ampR
4343..4982	ori

ATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTC  
CCTCTAGATCACAAGTTTGTACAAAAAAGCTGAACGAGAAACGTAAAATGATATAAATAT  
CAATATATTAAATTAGATTTTGCATAAAAAACAGACTACATAATACTGTAAACACAACA  
TATCCAGTCACTATGGCGGCCGATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGC  
TCGTATAATGTGTGGATTTTGTAGTATAGGATCCGGCGAGATTTTCAGGAGCTAAGGAAGCT  
AAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAA  
GAACATTTTGTGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCTGCTG  
GATATTACGGCCTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTATCCGGCCTTT  
ATTACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTCGTATGGCAATGAAAGAC  
GGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTACACCGTTTCCATGAGCAAAC  
GAAACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATA  
TATTCGCAAGATGTGGCGTGTACGGTGAAACCTGGCCTATTTCCCTAAAGGGTTTATT  
GAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTGTATTAAAC  
GTGGCCAATATGGACAACCTTCTTCGCCCCCGTTTTCACCATGGGCAAATATTATACGCAA  
GGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGGTTTCATCATGCCGTCTGTGATGGCTTC  
CATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCG  
TAAACGCGTGATCCGGCTTACTAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGAT  
TTTTGCGGTATAAGAATATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTG  
CTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCAAGGCAT  
ATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCGTCT  
GCGTGCCGAACGCTGGAAAGCGGAAATCAGGAAGGGATGGCTGAGGTGCGCCCGGTTTAT  
TGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGTGAAATGCAGTTTAAAGTTT  
ACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTG  
ACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGACGCTCTGCTGTCAGATAAAG  
TCTCCCGTGAACTTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCA  
CCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACC  
GCGAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTCAGGCT  
CCCTTATACACAGCCAGTCTGCAGGTCGACCATAGTGACTGGATATGTTGTGTTTTACAG  
TATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTT  
TACGTTTCTCGTTTCTGTTTCTTGTACAAAGTGGTGATTATGTCCCCTATACTAGGTTAT  
TGGAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGGAAATATCTTGAAGAAAA  
TATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAA  
TTGGGTTTGGAGTTTCCCAATCTTCTTATTATATTGATGGTGATGTTAAATTAACACAG  
TCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAA  
GAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCG  
AGAATTGCATATAGTAAAGACTTTGAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCT  
GAAATGCTGAAAATGTTTGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCAT  
GTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCA  
ATGTGCCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCA  
CAAATTGATAAGTACTTGAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAA  
GCCACGTTTGGTGGTGGCGACCATCTCCAAATCGGATCTGGTTCCGCGTCCATGGGGA  
TCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCGCTT  
CCCGATAAGGGAGCAGGCCAGTAAAGCATTACCCGTGGTGGGGTTCCCGAGCGGCCAAA  
GGGAGCAGACTCTAAATCTGCCGTCTCGACTTCGAAGGTTTGAATCCTTCCCCACCAC  
CATCACTTTCAAAGTGAATTCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAA-

FIGURE 96B



ACGGGTCTTGAGGGGTTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCACAGGACGG  
GTGTGGTCGCCATGATCGCGTAGTCGATAGTGGCTCCAAGTAGCGAAGCGAGCAGGACTG  
GGCGGCGGCCAAAGCGGTCCGACAGTGCTCCGAGAACGGGTGCGCATAGAAATTGCATCA  
ACGCATATAGCGCTAGCAGCACGCCATAGTGAAGTGGCGATGCTGTCGGAATGGACGATAT  
CCCGCAAGAGGCCCCGGCAGTACCGGCATAACCAAGCCTATGCCTACAGCATCCAGGGTGA  
CGGTGCCGAGGATGACGATGAGCGCATTGTTAGATTTTCATACACGGTGCCTGACTGCGTT  
AGCAATTTAACTGTGATAAACTACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACAT  
GAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATG  
ATAATAATGGTTTCTTAGACGTGAGTGGCAGTTTTCGGGGAAATGTGCGCGGAACCCCT  
ATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGA  
TAAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCC  
CTTATTCCCTTTTTTGGCGCATTTCCTGTTTTCGCTCAGGAGAAACGCTGGTG  
AAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTC  
AACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAAGCTTTCCAATGATGAGCACT  
TTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTGACGCCGGGCAAGAGCAACTC  
GGTCCGCCGATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG  
CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT  
AACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTT  
TTGCACAACATGGGGGATCATGTAACCTCGCTTGATCGTTGGGAACCGGAGCTGAATGAA  
GCCATACCAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGC  
AACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATG  
GAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATT  
GCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCA  
GATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGAT  
GAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCA  
GACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGG  
ATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCG  
TTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT  
CTGCGCGTAATCTGCTGCTTGCAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTG  
CCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCCAGCAGAGCGCAGATA  
CCAAATACTGTCTTCTAGTGATGCGGTAGTGGCCACCACTTCAAGAACTCTGTAGCA  
CCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAG  
TCGTGTCTTACCGGGTTGGAAGTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGC  
TGAACGGGGGGTTCTGTCACACAGCCAGCTTGGAGCGAAGACCTACACCGAACTGAGA  
TACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG  
TATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAAC  
GCCTGGTATCTTTATAGTCCTGTGCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTG  
TGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCTTTTTACGG  
TTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCTGATTCT  
GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGAGCCGAACGACC  
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTT  
ACGCATCTGTGCGGTATTTACACCCGCATATATGGTGCACCTCTCAGTACAATCTGCTCTG  
ATGCCGCATAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGACTGGGTCTATGGCTGC  
GCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATC  
CGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTGAGAGGTTTTACCGTC  
ATCACCGAAACGCGCGAGGCAGCTGCGGTAAAGCTCATCAGCGTGGTCGTGAAGCGATTCT  
ACAGATGTCTGCCTGTTTATCCGCGTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGT  
CTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTCTGTTTGGTCACTGATGC  
CTCCGTGTAAGGGGGATTCTGTTTATGGGGTAATGATACCGATGAAACGAGAGAGGAT  
GCTCACGATACGGGTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAA  
ACAACTGGCGGTATGGATGCGGCGGGACAGAGAAAATCACTCAGGGTCAATGCCAGCG  
CTTCGTTAATAACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCCTGCGATGCAGAT  
CCGGAACATAATGGTGCAGGGCGCTGACTTCCGCGTTTCCAGACTTTACGAAACACGGAA  
ACCGAAGACCATTCATGTTGTTGCTCAGGTGCGAGACGTTTTGCGAGCAGCAGTCGCTTCA  
CGTTTCGCTCGCGTATCGGTGATTCTGCTAACCAGTAAGGCAACCCCGCCAGCCTAG  
CCGGGTCTCAACGACAGGAGCAGATCATGCGCACCCGTGGCCAGGACCCAACGCTGCC  
CGAGATGCGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGG  
GTTGGTTTGCGCATTCACAGTTCTCCGCAAGAATTGATTGGCTCCAATTCTTGAGTGGT-

FIGURE 96C

GAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTGAGGTGAGGTGGCCCCGGCTCCATGCA  
CCGCGACGCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCCTACAATCCATGCCAAC  
CCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCCAGTGATC  
GAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCATCT  
ACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCGATGCCGCCGGAAGCGAGAAGA  
ATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGC  
GCGTCGGCCGCCATGCCGGCGATAATGGCCTGCTTCTCGCCGAAACGTTTGGTGGCGGGA  
CCAGTGACGAAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCAAGCGACAGGCCG  
ATCATCGTCGCGCTCCAGCGAAAGCGGTCTCGCCGAAAATGACCCAGAGCGCTGCCGGC  
ACCTGTCCTACGAGTTGCATGATAAAGAAGACAGTCATAAGTGCGGCGACGATAGTCATG  
CCCCGCGCCCAACGGAAGGAGCTGACTGGGTGAAGGCTCTCAAGGGCATCGGTGATCG  
ACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTT  
GAGCACCGCCGCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGC  
CACGGGGCCTGCCACCATAACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGCGGAGC  
CCGATCTTCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCC  
GGTGATGCCGGCCACGATGCGTCCGGCGTAGAGG

FIGURE 96A



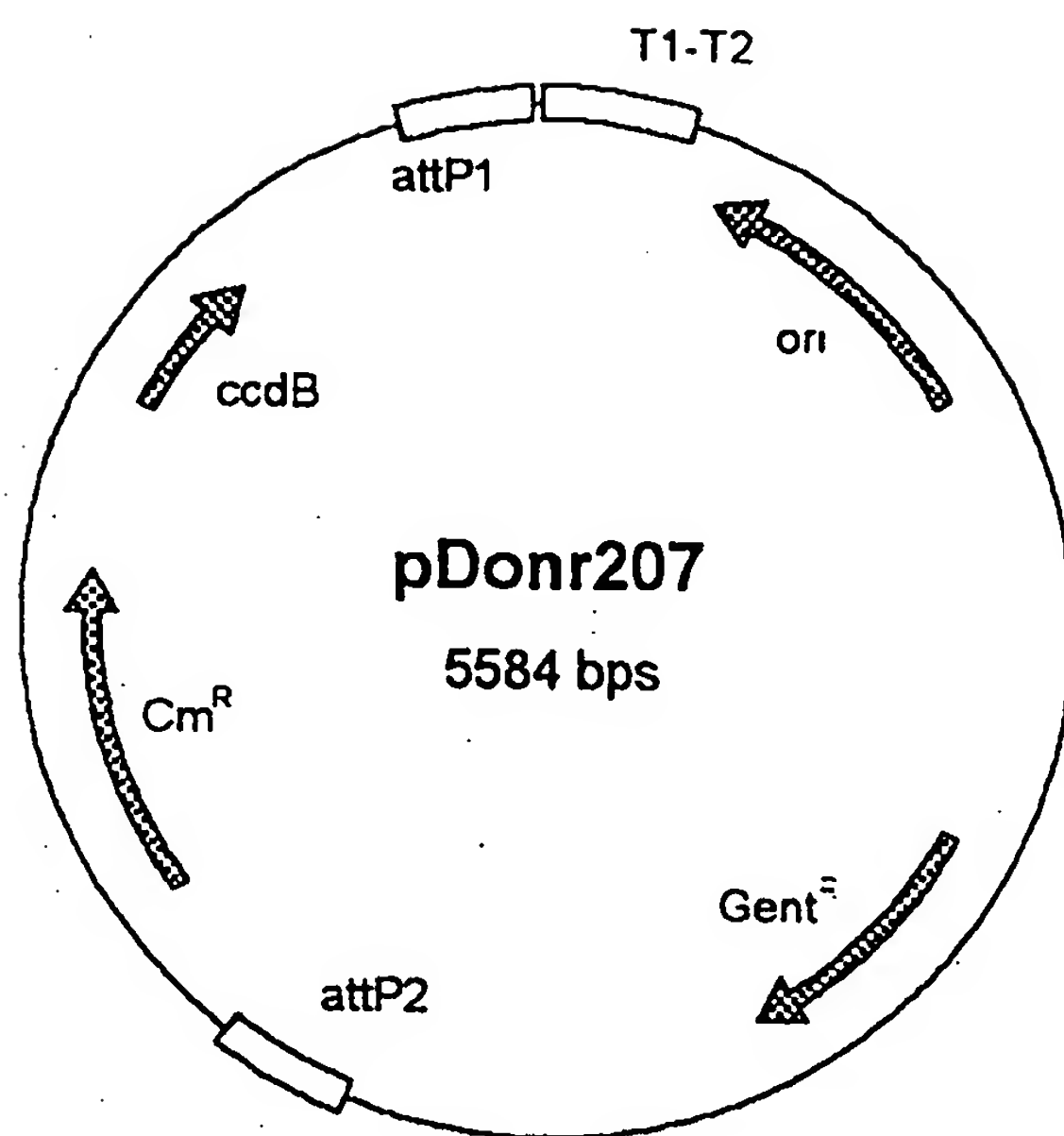


FIGURE 97A

pDONR207

5584 bp

GCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGGAAGACTGGGC  
CTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTCCTGAGTAGGACAAATCCGCCGGG  
AGCGGATTTGAACGTTGTGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGCCATA  
AACTGCCAGGCATCAAATAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGTTTCT  
ACAAACTCTTCTGGCTAGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGA  
AAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTG  
GCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAG  
AGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTC  
GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCG  
GGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTT  
CGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCGACCGCTGCGCCTTATCC  
GGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCC  
ACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG  
TGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCA  
GTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC  
GGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGAT  
CCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATT  
TTGGTCATGAGCTTGCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACC  
AATTAACCAATTCTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAATTTATTCA  
TATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACT  
CACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTC  
CAACATCAATACAACCTATTAGTAGCCAACCACTAGAACTATAGCTAGAGTCTTGGGCGA  
ACAAACGATGCTCGCCTTCCAGAAAACCGAGGATGCGAACCCTTCATCCGGGGTCAGCA  
CCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCGATCTCCTGAAGCCAGGGCAGATCCG  
TGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCAATGCCTGACGATGCGTGGAGA  
CCGAAACCTTGCGCTCGTTCCGACGCCAGGACAGAAATGCCTCGACTTCGCTGCTGCCCA  
AGGTTGCCGGGTGACGCACACCGTGGAACGGATGAAGGCACGAACCCAGTTGACATAAG  
CCTGTTCCGTTTCGTAAACTGTAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAA  
CCTTGACCGAACGCAGCGGTGGTAACGGCGCAGTGGCGGTTTTTCATGGCTTGTTATGACT  
GTTTTTTTTGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTC  
GATGTTTGATGTTATGGAGCAGCAACGATGTTACGCAGCAGCAACGATGTTACGCAGCAG  
GGCAGTCGCCCTAAACAAAGTTAGGTGGCTCAAGTATGGGCATCATTGCGACATGTAGG  
CTCGGCCCTGACCAAGTCAAATCCATGCGGGCTGCTCTTGATCTTTTCGGTCGTGAGTTC  
GGAGACGTAGCCACCTACTCCCAACATCAGCCGGACTCCGATTACCTCGGGAACCTTGCTC  
CGTAGTAAGACATTCATCGCGCTTGCTGCCTTCGACCAAGAAGCGGTTGTTGGCGCTCTC  
GCGGCTTACGTTCTGCCCAGGTTTGAGCAGCCGCGTAGTGAGATCTATATCTATGATCTC  
GCAGTCTCCGGCGAGCACCGGAGGCAGGGCATTGCCACCGCGCTCATCAATCTCCTCAAG  
CATGAGGCCAACGCGCTTGTTGCTTATGTGATCTACGTGCAAGCAGATTACGGTGACGAT  
CCCGCAGTGGCTCTCTATACAAAGTTGGGCATACGGGAAGAAGTGATGCACTTTGATATC  
GACCCAAGTACCGCCACCTAACAAATTCGTTCAAGCCGAGATCGGCTTCCCGGCCCTAATTT  
CCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGG  
TGAGAAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGGCCAGCCATTACG  
CTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTTCATTCGTGATTGCGCCTGAGC  
GAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCG  
GCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAA  
TACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGT  
ACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATCCGTGAGCCAGTTTAGTCTGAC  
CATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGG  
CGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCG  
AGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGT  
TTCCCGTTGAATATGGCTCATAACACCCCTGTATTACTGTTTATGTAAGCAGACAGTTT  
TATTGTTTCATGATGATATATTTTTATCTTGTCGAATGTAACATCAGAGATTTTGAGACAC  
GGGCCAGAGCTGCAGCTGGATGGCAAATAATGATTTTATTTTGACTGATAGTGACCTGTT  
CGTTGCAACAAATTGATAAGCAATGCTTTCTTATAATGCCAACTTTGTACAAGAAAGCTG  
AACGAGAAACGTAAATGATATAAATATCAATATATTAAATTAGATTTTGCATAAAAAAC  
AGACTACATAATACTGTAAAACACAACATATCCAGTCACTATGAATCAACTACTTAGATG-

FIGURE 97B

GTATTAGTGACCTGTAGTCGACTAAGTTGGCAGCATCACCCGACGCACTTTGCGCCGAAT  
AAATACCTGTGACGGAAGATCACTTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATA  
CCGGGAAGCCCTGGGCCAACTTTGGCGAAAATGAGACGTTGATCGGCACGTAAGAGGTTTC  
CAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTTGAGTTATCGAGATT  
TTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAAATCACTGGATATAACCACCGTTGATAT  
ATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTACCTA  
TAACCAGACCGTTTCAGCTGGATATTACGGCCTTTTAAAGACCGTAAAGAAAAATAAGCA  
CAAGTTTTATCCGGCCTTTATTACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATT  
CCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCAACCCTTGTTACAC  
CGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAGTGAATACCACGACGATTT  
CCGGCAGTTTCTACACATATATTGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTA  
TTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTT  
CACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCCGTTTTTCACCAT  
GGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTGAGGTTTCATCA  
TGCCGTCTGTGATGGCTTCCATGTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGA  
TGAGTGGCAGGGCGGGGCGTAATCGCGTGATCCGGCTTACTAAAAGCCAGATAACAGTA  
TGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATACTGATATGTATAACCGAAG  
TATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGC  
TATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACCAACCATGC  
AGAATGAAGCCCGTCGTCTGCGTGCCGAACGCTGGAAAGCGGAAAATCAGGAAGGGATGG  
CTGAGGTGCGCCGGTTTATTGAAATGAACGGCTCTTTTGCTGACGAGAACAGGGACTGGT  
GAAATGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGAT  
GTACAGAGTGATATTATTGACACGCCCGGGCGACGGATGGTGATCCCCCTGGCCAGTGCA  
CGTCTGCTGTCAGATAAAGTCTCCCGTGAACCTTTACCCGGTGGTGTCATATCGGGGATGAA  
AGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAA  
GTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGG  
GGAATATAAATGTCAGGCTCCCTTATACACAGCCAGTCTGCAGGTCGATACAGTAGAAAT  
TACAGAACTTTATCACGTTTAGTAAGTATAGAGGCTGAAAATCCAGATGAAGCCGAACG  
ACTTGTAAGAGAAAAGTATAAGAGTTGTGAAATTGTTCTTGATGCAGATGATTTTCAGGA  
CTATGACACTAGCGTATATGAATAGGTAGATGTTTTTATTTTGTACACAAAAAAGAGGC  
TCGCACCTCTTTTTCTTATTTCTTTTTATGATTTAATACGGCATTGAGGACAATAGCGAG  
TAGGCTGGATACGACGATTCCGTTTGAGAAGAACATTTGGAAGGCTGTCGGTCGACTAAG  
TTGGCAGCATCACCCGAAGAACATTTGGAAGGCTGTCGGTCGACTACAGGTCACTAATAC  
CATCTAAGTAGTTGATTATAGTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCT  
GTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTACGTTTCTCGTT  
CAGCTTTTTTGTACAAAGTTGGCATTATAAAAAAGCATTGCTCATCAATTTGTTGCAACG  
AACAGGTCATATCAGTCAAAATAAAATCATTATTTGGGGCCCGAGATCCATGCTAGCGT  
TAAC

FIGURE 97C

## pMAB85

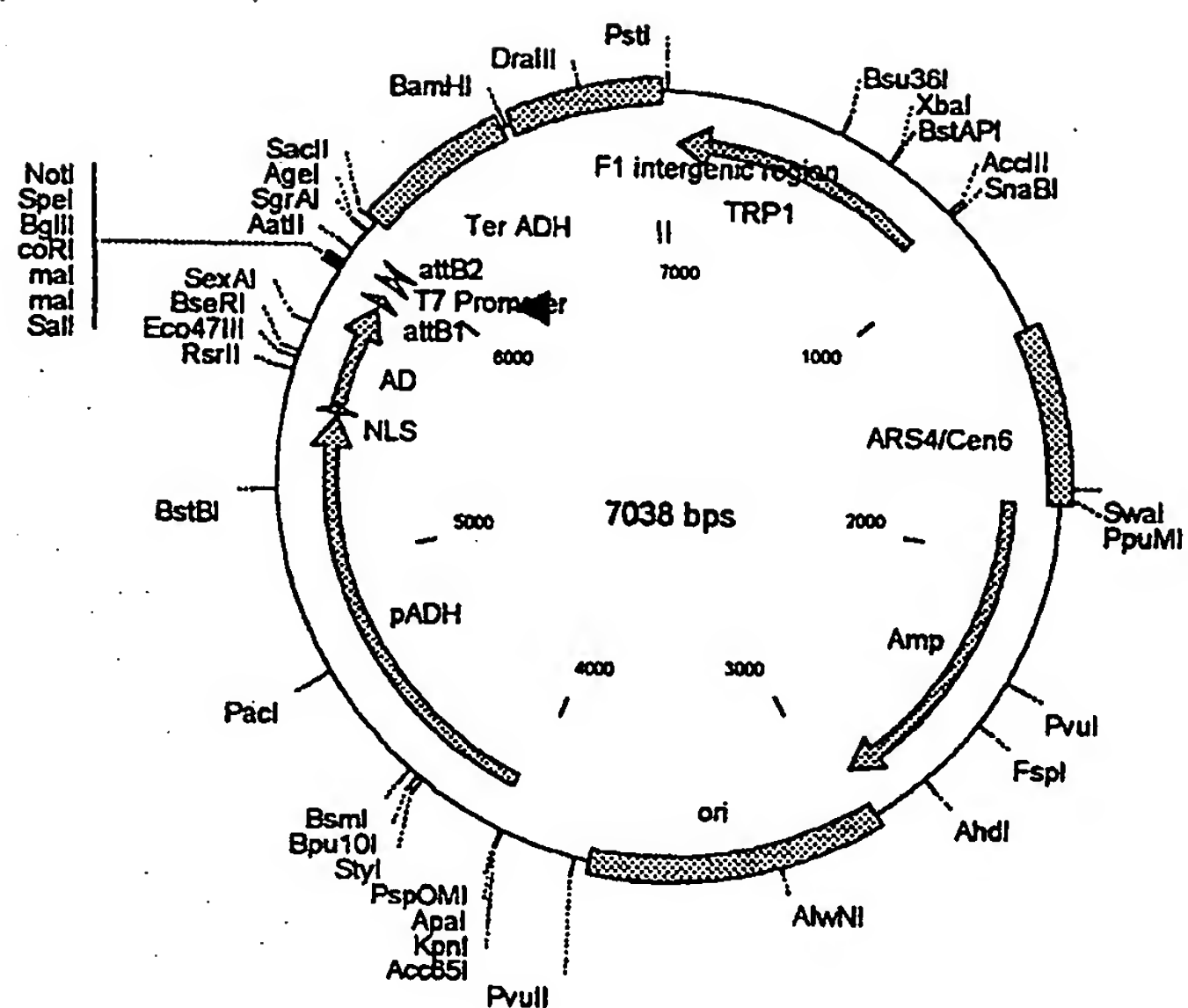


FIGURE 98A

pMAB85 7038 bp

GCCTTACGCATCTGTGCGGTATTTTCACACCGCAGGCAAGTGCACAAACAATACTTAAATA  
AATACTACTCAGTAATAACCTATTTCTTAGCATTTTTGTGCTATTTTGTAG  
AGTCTTTTACACCATTTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTA  
ATCTAAGCGCATCACCAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGC  
TTTCGGGGCTCTCTTGCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTCAC  
CTGTCCCACCTGCTTCTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTG  
CACTGAGTAGTATGTTGTCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGA  
GGAACCTTTGGTATTCTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGT  
AATCATTGACCAGAGCCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGT  
ATTTTCGGAGTGCCTGAACTATTTTATATGCTTTTACAAGACTTGAAATTTTCCTTGCAA  
TAACCGGGTCAATTGTTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCAT  
CGGAATCTAGAGCACATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACCTTTCACCAATG  
GACCAGAACTACCTGTGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAA  
TCACGTATACTCACGTGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTC  
TTTTTTTCGACCGAATTAATTCTTAATCGGCAAAAAAAGAAAAGCTCCGGATCAAGATTGT  
ACGTAAGGTGACAAGCTATTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTC  
ATAACTGCAAAGTACACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATA  
TATAGTAATGTCGTTTATGGTGCACCTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAA  
GCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGG  
CATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCAC  
CGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCCTATTTTATAGGTTA  
ATGTCATGATAATAATGGTTTCTTAGGACGGATCGCTTGCTGTAACTTACACGCGCCTC  
GTATCTTTTAATGATGGAATAATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTT  
TGTATTTGGATTTTAGAAAGTAAATAAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAA  
AAATAAACAAAGGTTTAAAAAATTTCAACAAAAGCGTACTTTACATATATATTTATTAG  
ACAAGAAAAGCAGATTAAATAGATATACATTCGATTAAACGATAAGTAAATGTAAATCA  
CAGGATTTTCGTGTGTGGTCTTCTACACAGACAAGATGAAACAATTCGGCATTAAATACCT  
GAGAGCAGGAAGAGCAAGATAAAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTA  
CATCTTCGGAAAACAAAACCTATTTTCTTTAATTTCTTTTCTTACTTTCTATTTTAA  
TTTATATATTTATATTAATAAATTTAAATTATAATTATTTTATAGCACGTGATGAAAAG  
GACCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAA  
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCTGATAAATGCTTCAATAATAT  
TGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTCGCG  
GCATTTTGCCTTCCCTGTTTTCGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAA  
GATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTT  
GAGAGTTTTCGCCCCGAAGAACGTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT  
GGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTAT  
TCTCAGAATGACTTGGTTGAGTACTCACCAAGTCACAGAAAAGCATCTTACGGATGGCATG  
ACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTA  
CTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTCACAACATGGGGGAT  
CATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAG  
CGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAAGTGGCGAA  
CTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCA  
GGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCC  
GGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGT  
ATCGTAGTTATCTACACGACGGGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATC  
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATAT  
ATACTTTAGATTGATTTAAAACCTTCATTTTAAATTTAAAAGGATCTAGGTGAAGATCCTT  
TTTGATAATCTCATGACCAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGAC  
CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGC  
TTGCAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCA  
ACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTA  
GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCT  
CTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTG  
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGCTGAACGGGGGGTTCTGTC-

Figure 98B



ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCAT  
TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG  
GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGT  
CCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGG  
CCGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCTGG  
CCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC  
GCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAGTG  
AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATT  
CATTAATGCAGCTGGCAGCAGAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA  
ATTAATGTGAGTTACCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCT  
CCTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCAT  
GATTACGCCAAGCTCGGAATTAACCTCACTAAAGGGAACAAAAGCTGGGTACCGGGCCC  
CCCCTCGAGATCCGGGATCGAAGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATG  
AAGGCAAAAGACAAATATAAGGGTCGAACGAAAAATAAAGTGAAAAGTGTTGATATGATG  
TATTTGGCTTTGCGGCGCCGAAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCT  
GTGGCGGACCCGCGCTCTTGCCGGCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGGC  
GGAGTTTTCGCGCTGCATTTTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGA  
AGCAATAAGAATGCCGTTGGGGTTGCGATGATGACGACCACGACAACCTGGTGTCAATTAT  
TTAAGTTGCCGAAAGAACCTGAGTGCATTTGCAACATGAGTATACTAGAAGAATGAGCCA  
AGACTTGCGAGACGCGAGTTTGCCGGTGGTGCGAACAATAGAGCGACCATGACCTTGAAG  
GTGAGACGCGCATAACCGCTAGAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCA  
GTATAAATAGACAGGTACATAACAACCTGGAAATGGTGTCTGTTTGAGTACGCTTTCAA  
TTCATTTGGGTGTGCACTTTATTATGTTACAATATGGAAGGGAACCTTACACTTCTCCTA  
TGCACATATATTAATTAAAGTCCAATGCTAGTAGAGAAGGGGGGTAAACCCCCTCCGCGC  
TCTTTTCCGATTTTCTTAAACCGTGGAATATTTCCGATATCCTTTTGTTGTTTCCGGG  
TGTACAATATGGACTTCCTCTTTCTGGCAACCAAACCCATACATCGGGATTCCCTATAAT  
ACCTTCGTTGGTCTCCCTAACATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATA  
CCAGACAAGACATAATGGGCTAAACAAGACTACACCAATTACACTGCCTCATTGATGGTG  
GTACATAACGAACTAATACTGTAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTC  
ACTACCCTTTTCCATTTGCCATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTC  
TTTTTTTTTCTTTTCTCTCTCCCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAA  
ATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCACAGATGTCGTTG  
TTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAACTTTTTCTTCTTCATTACAG  
CACACTACTCTCTAATGAGCAACGGTATACGGCCTTCCTTCCAGTTACTTGAATTTGAAA  
TAAAAAAAGTTTGCCGCTTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTG  
TTTCTCGTCATTGTTCTCGTTCCCTTTCTTCTTCTTTCTGACAAATATTTCA  
AGCTATACCAAGCATAACAATCAACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCG  
AGCGGCGCCAATTTTAATCAAAGTGGGAATATTGCTGATAGCTCATTGTCCTTCACTTTC  
ACTAACAGTAGCAACGGTCCGAACCTCATAACAACCTCAAACAAATTCTCAAGCGCTTTCA  
CAACCAATTGCCTCCTCTAACGTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGT  
AAAATTGATGATGGTAATAATTCAAACCACTGTACCTGGTTGGACGGACCAAACCTGCG  
TATAACGCGTTTGGAATCACTACAGGGATGTTTAATACCACTACAATGGATGATGTATAT  
AACTATCTATTGATGATGAAGATACCCACCAACCCAAAAAAGAGGGTGGGTGCGATC  
ACAAGTTTGTACAAAAAGCAGGCTTGTCGACCCCGGGAATTGAGATCTACTAGTGCGGC  
CGCACGCGTACCCAGCTTTCTTGTAACAAGTGGTGACGTCGAGCTCCCTATAGTGAGTCG  
TATTACACTGGCCGTCGTTTACAACGTCGTGACTGGGAAACACCGGTGAGCTCTAAGT  
AAGTAACGGCCGCCACCGCGGTGGAGCTTTGGACTTCTTCGCCAGAGGTTTGGTCAAGTC  
TCCAATCAAGGTTGTGCGCTTGCTACCTTGCCAGAAATTTACGAAAAGATGGAAAAGGG  
TCAAATCGTTGGTAGATACGTTGTTGACACTTCTAAATAAGCGAATTTCTTATGATTTAT  
GATTTTATTATTAAATAAGTTATAAAAAAATAAGTGATACAAATTTTAAAGTGACTC  
TTAGGTTTAAACGAAATTTCTTGTCTTGAGTAACTCTTCTGTAGGTCAGGTTGCT  
TTCTCAGGTATAGCATGAGGTCGCTCTATTGACCACACCTCTACCGGCATGCCGAGCAA  
ATGCCTGCAAATCGCTCCCATTTACCCCAATTGTAGATATGCTAACTCCAGCAATGAGT  
TGATGAATCTCGGTGTGTATTTATGTCCTCAGAGGACAATACCTGTTGTAATCGTTCTT  
CCACACGGATCCGCATCAGGCGAAATTGTAAACGTTAATATTTTGTTAAATTCGCGTTA  
AATATTTGTTAAATCAGCTCATTTTAAACCAATAGGCCGAAATCGGCAAAATCCCTTAT  
AAATCAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAAACAAGAGTCCA  
CTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGC-



CCACTACGTGAACCATCACCCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTA  
AATCGGAACCCTAAAGGGAGCCCCGATTAGAGCTTGACGGGGAAAGCCGGCGAACGTG  
GCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCG  
GTCACGCTGCGCGTAACCACACCCGCGCGCTTAATGCGCCGCTACAGGGCGCGTCC  
CATTCGCCATTCACTGCA

FIGURE 98D

pMAB86

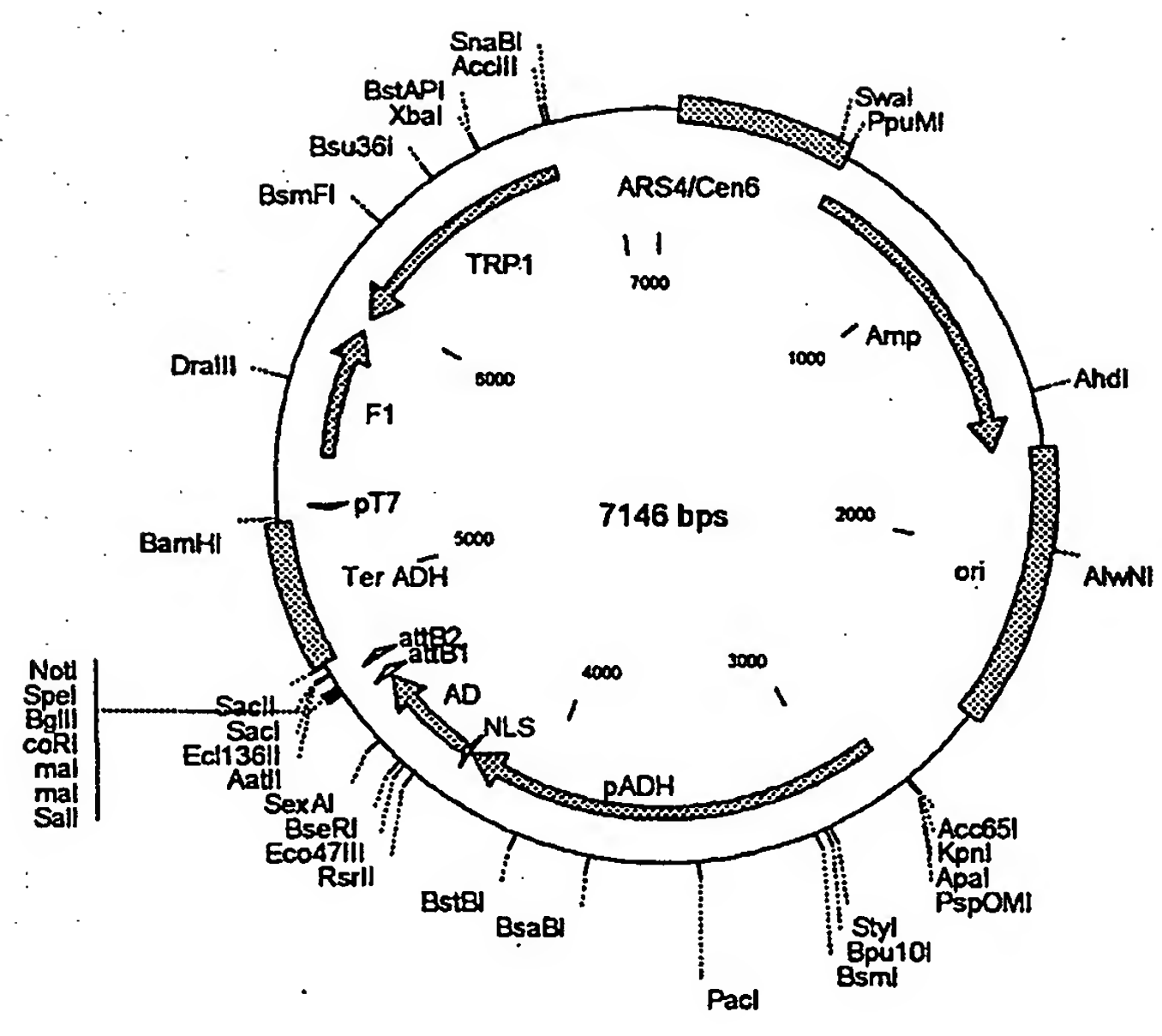


FIGURE 99A

pMAB86 7146 bp

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT  
CTTAGGACGGATCGCTTGCCCTGTAACCTACACGCGCCTCGTATCTTTTAATGATGGAATA  
ATTTGGGAATTTACTCTGTGTTTATTTATTTTATGTTTTGTATTTGGATTTTAGAAAGT  
AAATAAGAAGGTAGAAGAGTTACGGAATGAAGAAAAAAATAAACAAAGGTTTAAAAA  
ATTTCAACAAAAAGCGTACTTTACATATATATTTATTAGACAAGAAAAGCAGATTAAATA  
GATATACATTGATTAACGATAAGTAAAATGTAAAATCACAGGATTTTCGTGTGTGGTCT  
TCTACACAGACAAGATGAAACAATTCGGCATTAATACCTGAGAGCAGGAAGAGCAAGATA  
AAAGGTAGTATTTGTTGGCGATCCCCCTAGAGTCTTTTACATCTTCGGAAAACAAAACT  
ATTTTTTCTTTAATTTCTTTTTTTTACTTTCTATTTTAAATTTATATATTTATATTAAAA  
ATTTAAATTATAATTATTTTATAGCACGTGATGAAAAGGACCCAGGTGGCACTTTTCGG  
GGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCG  
CTCATGAGACAATAACCCCTGATAAATGCTTCAATAATATTGAAAAGGAAGAGTATGAGT  
ATTCAACATTTCCGTGTGCGCCCTTATTCCTTTTTTTCGCGGCATTTTGCCTTCCTGTTTTT  
GCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG  
GGTTACATCGAACTGGATCTCAACAGCGGTAAAGATCCTTGAGAGTTTTTCGCCCCGAAGAA  
CGTTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATT  
GACGCCGGGCAAGAGCAACTCGGTGCGCGCATACTATTCTCAGAATGACTTGGTTGAG  
TACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGT  
GCTGCCATAACCATGAGTGATAAAGTGGGCAACTTACTTCTGACAACGATCGGAGGA  
CCGAAGGAGCTAACCGCTTTTTTTCACAACATGGGGGATCATGTAAGTTCGCTTGATCGT  
TGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTA  
GCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGG  
CAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCC  
CTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT  
ATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACG  
GGCAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTG  
ATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAA  
CTTCATTTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAA  
ATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGA  
TCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG  
CTACCAGCGGTGGTTTGTGTTGCGCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGT  
GGCTTCAGCAGAGCGCAGATAACCAATACTGTCTTCTAGTGATAGCCGTAGTTAGGCCAC  
CACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTG  
GCTGCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCG  
GATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGA  
ACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATTGAGAAAGCGCCACGCTTCCC  
GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAGC  
AGGGAGCTTCCAGGGGGGAACGCCTGGTATCTTTATAGTCTGTGCGGGTTTCGCCACCTC  
TGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGAGCCTATGGAAAAACGCC  
AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTT  
CCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC  
GCTCGCCGACCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGC  
CCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTATTAATGCAGCTGGCACGAC  
AGGTTTTCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTACCTCACT  
CATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCCTATGTTGTGTGGAATTGTG  
AGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCGGAATT  
AACCCTCACTAAAGGGGAACAAAAGCTGGGTACCGGGCCCCCTCGAGATCCGGGATCGA  
AGAAATGATGGTAAATGAAATAGGAAATCAAGGAGCATGAAGGCAAAAGACAAATATAAG  
GGTCGAACGAAAAATAAAGTGAAAAGTGTTGATATGATGATTTGGCTTTGCGGCGCCGA  
AAAAACGAGTTTACGCAATTGCACAATCATGCTGACTCTGTGGCGGACCCGCGCTCTTGC  
CGGCCCGGCGATAACGCTGGGCGTGAGGCTGTGCCCGGCGGAGTTTTTTGCGCCTGCATT  
TTCCAAGGTTTACCCTGCGCTAAGGGGCGAGATTGGAGAAGCAATAAGAATGCCGGTTGG  
GGTTGCGATGATGACGACCACGACAACCTGGTGTCTATTATTTAAGTTGCCGAAAGAACCTG  
AGTGCAATTTGCAACATGAGTATACTAGAAGAATGAGCCAAGACTTGCGAGACGCGAGTTT  
GCCGGTGGTGCGAACAAATAGAGCGACCATGACCTTGAAGGTGAGACGCGCATAACCGCTA-

FIGURE 99B

GAGTACTTTGAAGAGGAAACAGCAATAGGGTTGCTACCAGTATAAATAGACAGGTACATA  
CAACACTGGAAATGGTTGTCTGTTTGAGTACGCTTTCATTTGGGTGTGCACTTTA  
TTATGTTACAATATGGAAGGGAACCTTACACTTCTCCTATGCACATATATTAATTAAAGT  
CCAATGCTAGTAGAGAAGGGGGGTAAACCCCCCTCCGCGCTCTTTTCCGATTTTTTTCTAA  
ACCGTGGAATATTTCCGATATCCTTTTGTGTTTCCGGGTGTACAATATGGACTTCCTCT  
TTTCTGGCAACCAAACCCATACATCGGGATTCTATAATACCTTCGTTGGTCTCCCTAAC  
ATGTAGGTGGCGGAGGGGAGATATACAATAGAACAGATACCAGACAAGACATAATGGGCT  
AAACAAGACTACACCAATTACACTGCCTCATTTGATGGTGGTACATAACGAACATAACTG  
TAGCCCTAGACTTGATAGCCATCATCATATCGAAGTTTCACTACCCTTTTTCCATTGCCC  
ATCTATTGAAGTAATAATAGGCGCATGCAACTTCTTTTCTTTTTTTTTCTTTTCTCTCTC  
CCCCGTTGTTGTCTCACCATATCCGCAATGACAAAAAATGATGGAAGACACTAAAGGA  
AAAAATTAACGACAAAGACAGCACCAACAGATGTCGTTGTTCCAGAGCTGATGAGGGGTA  
TCTTCGAACACACGAAACTTTTTCTTCTTTCATTACGCACACTACTCTCTAATGAGCA  
ACGGTATACGGCCTTCTTCCAGTTACTTGAATTTGAAATAAAAAAGTTTGCCGCTTTG  
CTATCAAGTATAAATAGACCTGCAATTATTAATCTTTTGTTCCTCGTCATTGTTCTCGT  
TCCCTTTCTTCTTGTCTTTCTTTTCTGCACAATATTTCAAGCTATACCAAGCATAACAATC  
AACTCCAAGCTTATGCCCAAGAAGAAGCGGAAGGTCTCGAGCGGCGCAATTTTAATCAA  
AGTGGGAATATTGCTGATAGCTCATTGTCCTTCACTTTCACCTAACAGTAGCAACGGTCCG  
AACCTCATAACAACTCAAACAAATTCTCAAGCGCTTTCACAACCAATTGCCTCCTCTAAC  
GTTTCATGATAACTTCATGAATAATGAAATCACGGCTAGTAAAATTGATGATGGTAATAAT  
TCAAAACCACTGTCACCTGGTTGGACGGACCAAACTGCGTATAACGCGTTTGGAATCACT  
ACAGGGATGTTTAATACCCTACAATGGATGATGTATATAACTATCTATTGATGATGAA  
GATACCCACCAAACCCAAAAAAGAGGGTGGGTGCGATCACAAGTTTGTACAAAAAGCA  
GGCTTGTGCGACCCCGGAATTCAGATCTACTAGTGCGGCGCCACCGCGGTGGAGCTTTCT  
TGTAACAAGTGGTGACGTCGAGCTCTAAGTAAGTAACGGCCGCGCCACCGCGGTGGAGCTTT  
GGACTTCTTCGCCAGAGGTTTGGTCAAGTCTCCAATCAAGGTTGTCGGCTTGTCTACCTT  
GCCAGAAATTTACGAAAAGATGGAAAAGGGTCAAATCGTTGGTAGATACGTTGTTGACAC  
TTCTAAATAAGCGAATTTCTTATGATTTATGATTTTTATTATTAAATAAGTTATAAAAA  
AATAAGTGTATACAAATTTTAAAGTGACTCTTAGGTTTTAAACGAAAATTCTTGTTCTT  
GAGTAACTCTTTCCTGTAGGTGAGGTTGCTTCTCAGGTATAGCATGAGGTCGCTCTTAT  
TGACCACACCTCTACCGGCATGCCGAGCAAATGCCTGCAAATCGCTCCCATTTTACCCA  
ATTGTAGATATGCTAACTCCAGCAATGAGTTGATGAATCTCGGTGTGTATTTTATGTCCT  
CAGAGGACAATACCTGTTGTAATCGTTCTTCCACACGGATCCCAATTCGCCCTATAGTGA  
GTCGTATTACAATTCAGTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGT  
TACCCAACCTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGA  
GGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGACGCGCCC  
TGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT  
GCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTCTTCCCTTCTTCTCGCCACGTTTCGCC  
GGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTA  
CGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACAGTAGTGGGCCATCGCCC  
TGATAGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTG  
TTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTATAAGGGATT  
TTGCCGATTTCCGGCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAAT  
TTTAACAAAATATTAACGTTTACAATTTCTGTATGCGGTATTTTCTCCTTACGCATCTGT  
GCGGTATTTACACCGCAGGCAAGTGCACAAACAATACTTAATAAATACTACTCAGTAA  
TAACCTATTTCTTAGCATTTTTTGACGAAATTTGCTATTTTGTAGAGTCTTTTACACCAT  
TTGTCTCCACACCTCCGCTTACATCAACACCAATAACGCCATTTAATCTAAGCGCATCAC  
CAACATTTTCTGGCGTCAGTCCACCAGCTAACATAAAATGTAAGCTTTCGGGGCTCTCTT  
GCCTTCCAACCCAGTCAGAAATCGAGTTCCAATCCAAAAGTTACCTGTCCACCTGCTT  
CTGAATCAAACAAGGGAATAAACGAATGAGGTTTCTGTGAAGCTGCACTGAGTAGTATGT  
TGCAGTCTTTTGGAAATACGAGTCTTTTAATAACTGGCAAACCGAGGAACTCTTGGTATT  
CTTGCCACGACTCATCTCCATGCAGTTGGACGATATCAATGCCGTAATCATTGACCAGAG  
CCAAAACATCCTCCTTAGGTTGATTACGAAACACGCCAACCAAGTATTTCCGGAGTGCCTG  
AACTATTTTTATATGCTTTTACAAGACTTGAAATTTTCTTGCAATAACCGGGTCAATTG  
TTCTCTTTCTATTGGGCACACATATAATACCCAGCAAGTCAGCATCGGAATCTAGAGCAC  
ATTCTGCGGCCTCTGTGCTCTGCAAGCCGCAAACTTTCACCAATGGACCAGAACTACCTG  
TGAAATTAATAACAGACATACTCCAAGCTGCCTTTGTGTGCTTAATCACGTATACTCACG  
TGCTCAATAGTCACCAATGCCCTCCCTCTTGGCCCTCTCCTTTTCTTTTTTCGACCGAAT-

TAATTCCTTAATCGGCAAAAAAAGAAAAGCTCCGGATCAAGATTGTACGTAAGGTGACAAG  
CTATTTTTCAATAAAGAATATCTTCCACTACTGCCATCTGGCGTCATAACTGCAAAGTAC  
ACATATATTACGATGCTGTCTATTAAATGCTTCCTATATTATATATATAGTAATGTCGTT  
TATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACC  
CGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC  
AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCATCACCGAAAC  
GCGCGA

FIGURE 99D

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM  
(PCT Rule 13bis) REC'D

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>8</u> .	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input checked="" type="checkbox"/>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit February 27, 1999	Accession Number NRRL B-30103
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
Escherichia coli DB3.1(pEZC15101)	
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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Authorized officer <i>B. Indici</i>	Authorized officer



**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit <b>February 27, 1999</b>	Accession Number <b>NRRL B-30100</b>
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-1A)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30102
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-3C) In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>55</u> , line <u>16</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30101
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pENTR-2B)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p style="text-align: center;">For receiving Office use only</p> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <input checked="" type="checkbox"/> This sheet was received with the international application         </div> <div style="border: 1px solid black; padding: 5px;">           Authorized officer  </div>	<p style="text-align: center;">For International Bureau use only</p> <div style="border: 1px solid black; padding: 5px; margin-bottom: 5px;"> <input type="checkbox"/> This sheet was received by the International Bureau on:         </div> <div style="border: 1px solid black; padding: 5px; height: 40px;">           Authorized officer         </div>
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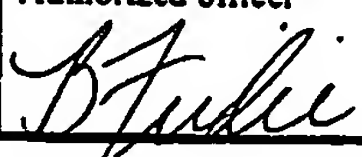
**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>WIPO</u> <u>PCT</u> <u>20-21</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30108
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB10B(pCMVSPORT6)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <div style="text-align: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></div>	
Name of depositary institution  Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30105
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <div style="text-align: right;">This information is continued on an additional sheet <input type="checkbox"/></div>	
Escherichia coli DB3.1(pEZC15103)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>54</u> , line <u>9</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></span>	
Name of depositary institution	
Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)	
1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30104
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
Escherichia coli DB3.1(pEZC15102)	
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer 	Authorized officer



**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM**  
(PCT Rule 13bis)

<b>A.</b> The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>31</u> .	
<b>B. IDENTIFICATION OF DEPOSIT</b> <div style="text-align: right;">Further deposits are identified on an additional sheet <input checked="" type="checkbox"/></div>	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country)  1815 N. University Street Peoria, Illinois 61604 United States of America	
Date of deposit    February 27, 1999	Accession Number    NRRL B-30099
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <div style="text-align: right;">This information is continued on an additional sheet <input type="checkbox"/></div>	
Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)  In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the international Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<p align="center"><b>For receiving Office use only</b></p> <div style="border: 1px solid black; padding: 5px;"> <input checked="" type="checkbox"/> This sheet was received with the international application         </div> <div style="border: 1px solid black; padding: 5px;">           Authorized officer    Barbara Fridie <i>BF</i>            PCT Operations - IPRD Team 1            703/305-3757    703/305-3230 (FA)         </div>	<p align="center"><b>For International Bureau use only</b></p> <div style="border: 1px solid black; padding: 5px;"> <input type="checkbox"/> This sheet was received by the International Bureau on:         </div> <div style="border: 1px solid black; padding: 5px;">           Authorized officer         </div>
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*Escherichia coli* DB3.1(pENTR-3C)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-3C)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-2B)**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pENTR-2B)**ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

**SINGAPORE**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-2B)

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.



*Escherichia coli* DB3.1(pENTR-1A)

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pENTR-1A)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pENTR-1A)**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)***AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**CANADA**

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**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

**FINLAND**

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*Escherichia coli DB3.1(pAHPKan) or Escherichia coli DB3.1(pAttPKan)*

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### CANADA

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#### DENMARK

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#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKan)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

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#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.



*Escherichia coli* DB3.1(pAHPKan) or *Escherichia coli* DB3.1(pAttPKa $\lambda$ )

#### SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB10B(pCMVSPORT6)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli DB10B(pCMVSPORT6)*

#### **SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

#### **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

*Escherichia coli* DB3.1(pEZC15103)

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pEZC15103)

#### ICELAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### NORWAY

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#### SINGAPORE

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for international publication of the application.

***Escherichia coli DB3.1(pEZC15103)*****SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent office or any person approved by the applicant in the individual case.

**UNITED KINGDOM**

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*Escherichia coli* DB3.1(pEZC15102)

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### CANADA

The applicant hereby requests that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the furnishing of a sample of deposited biological material referred to in the application only be effected to an independent expert nominated by the Commissioner of Patents.

#### DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent office or any person approved by the applicant in the individual case.

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Registration), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the National Board of Patents and Registration or any person approved by the applicant in the individual case.

*Escherichia coli* DB3.1(pEZC15102)

#### **ICELAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Icelandic Patent Office), or has been finally decided upon by the Icelandic Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### **NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in Rule 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

#### **NORWAY**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Norwegian Patent office or any person approved by the applicant in the individual case.

#### **SINGAPORE**

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*Escherichia coli* DB3.1(pEZC15102)

#### **SWEDEN**

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*Escherichia coli* DB3.1(pEZC15101)

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*Escherichia coli* DB3.1(pEZC15101)

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*Escherichia coli* DB3.1(pEZC15101)

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*Escherichia coli* DB3.1(pENTR-3C)

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/05432

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/91.2, 252.3, 320.1; 530/350; 536/ 23.1, 24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P --- Y,P	US 5,888,732 A (HARTLEY et al.) 30 March 1999, see entire document.	1-21, 25-30 36-38 ----- 22-24, 31-35
X - Y	HASAN et al. Escherichia coli genome targeting, I. Cre-lox-mediated in vitro generation of ori- plasmids and their in vivo chromosomal integration and retrieval. Gene. 1994, Vol. 150, pages 51-56, see entire document.	1-5, 10, 11, 19-21 ----- 15-18, 22-38
X - Y	KATZ et al. Site-specific recombination in Escherichia coli between the att sites of plasmid pSE211 from Saccharopolyspora erythraea. Mol. Gen. Genet. 1991, Vol. 227, pages 155-159, see entire document.	1-11, 19-21 ----- 15-18, 22-38

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search  
08 MAY 2000

Date of mailing of the international search report  
23 MAY 2000

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/05432

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	ASTUMIAN et al. Site-specific recombination between cloned attP and attB sites from the Haemophilus influenzae bacteriophage HP1 propagated in recombination deficient Escherichia coli. J of Bacteriology. March 1989, Vol. 171, No. 3, pages 1747-1750, see entire document.	1-11, 19-21 ----- 15-18, 22-38

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/05432

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07H 21/04; C07K 1/00, 14/00; C12N 1/21, 15/00, 15/09, 15/63, 15/70; C12P 19/34

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, STN (CAPLUS); DIALOG (MEDLINE, BIOSIS, SCISEARCH, PASCAL)

Terms: att (B?, P?, R?, L?), MCS, POLYLINKER, PLASMID, VECTOR, LOCALIZATION, SIGNAL, TRANSCRIPTION, TERMIN?, TRANSLATION?, ORI, REPLICON, GST, HEXHIST?, THIOREDOX?, CLEAVAGE, SITE?, SPECIF?, DIRECT?, RECOMBIN?, CLON?, INSERT?